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(54) SYSTEME D'OXYDATION ET DE BLANCHIMENT
COMPORTANT DES AGENTS D'OXYDATION PRODUITS
PAR ACTION ENZYMATIQUE

(54) OXIDATION AND BLEACHING SYSTEM WITH
ENZYMATICALLY PRODUCED OXIDIZING AGENTS

(57) L'invention concerne un système d'oxydation et de blanchiment comportant des agents d'oxydation produits par action enzymatique, à savoir un système enzymes-composants utilisé comme système d'oxydation et de blanchiment pour la production d'agents d'oxydation spéciaux hautement sélectifs, constitué de: a) composant de système (1): au moins une hydrolase de la classe d'enzymes 3.1, 3.1.1, 3.1.2, 3.1.3, 3.1.4 ou 3.1.7 et/ou au moins une hydrolase de la classe d'enzymes 3.5, 3.5.1, 3.5.2, 3.5.3, 3.5.4, 3.5.5 ou 3.5.99; b) composant de système (2): au moins un acide gras, de préférence ayant 6 à 26 atomes de C (saturé, mono ou polyinsaturé); c) composant de système (3): au moins un agent d'oxydation précurseur destiné à réagir avec les enzymes; d) composant de système (4): au moins une cétone du groupe des composés carbonylés.

(57) The invention relates to an oxidation and bleaching system with enzymatically produced oxidizing agents, namely an enzyme component system (ECM) as an oxidation and bleaching system for the production of special highly selective oxidizing agents, consisting of a) system components 1) at least one hydrolase from the enzyme class 3.1, 3.1.1, 3.1.2, 3.1.3, 3.1.4 or 3.1.7 and/or at least one hydrolase from the enzyme class 3.5, 3.5.1, 3.5.2, 3.5.3, 3.5.4, 3.5.5 or 3.5.99; b) system components 2) at least one fatty acid, preferably containing C₆ to C₂₆ (saturated, monounsaturated or polyunsaturated); c) system components 3) at least one precursor oxidizing agent for reaction with the enzymes, and d) system components 4) at least one ketone from the group of the carbonyl compounds.

PATENT CLAIMS

1. **Enzyme component system (ECS) as an oxidation and bleaching system for the preparation of special highly selective oxidants, consisting of**

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a) system component 1): at least one hydrolase from enzyme classes 3.1, 3.1.1, 3.1.2, 3.1.3, 3.1.4, or 3.1.7 and/or at least one hydrolase from enzyme classes 3.5, 3.5.1, 3.5.2, 3.5.3, 3.5.4, 3.5.5 or 3.5.99,

10 **b) system component 2): at least one fatty acid, preferably C₆ to C₂₆ (saturated, monounsaturated or polyunsaturated),**

c) system component 3): at least one oxidant precursor for reaction with the enzymes,

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d) system component 4): at least one ketone from the group of carbonyl compounds.

20 **2. Enzyme component system according to Claim 1, characterized in that enzymes of class 3.1.1.3 lipases (triacylglycerol lipase, triglyceracyl hydrolases) are used as system component 1).**

25 **3. Enzyme component system according to Claims 1 and 2, characterized in that enzymes of class 3.5.1.4, amidases, and/or class 3.5.5.1, nitrilases, are used as system component 1).**

30 **4. Enzyme component system according to Claims 1 and 2, characterized in that the enzymes of class 3.1.1.3 (lipases) are obtained from organisms such as Candida antarctica, Candida rugosa, Candida lipolytica, Candida cylindraceae, Candida spec., Geotrichum candidum, Humicula lanuginosa, Penicillium cambertii, Penicillium roquafortii, Aspergillus spec., Mucor javanicus, Mucor mehei, Rhizopus arrhizus,**

Rhizopus niveus, Rhizopus delamar, Rhizopus spec., Chromobacterium viscosum, Pseudomonas cepacia and Pseudomonas spec. from wheat seedlings or pancreas (pig or other sources) and from other sources.

5. Enzyme component system according to Claims 1 and 3, characterized in that the enzymes of classes 3.5.1.4 and 3.5.5.1 are obtained from organisms such as Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas acidovorus, Pseudomonas spec., Aspergillus nidulans, Aspergillus spec., Brevibacterium spec., Streptococcus pneumoniae, Rhodococcus spec. and others.
10. Enzyme component system according to Claims 1 through 5, characterized in that it contains enzymes from fungi, bacteria, animals or plants obtained from natural organisms or organisms modified by genetic engineering.
15. 7. Enzyme component system according to Claims 1 through 6, characterized in that modified enzymes, enzyme constituents, prosthetic groups or mimicking substances are used as catalysts.
20. 8. Enzyme component system according to Claims 1 through 7, characterized in that it contains as system component 2) one or more saturated, monounsaturated or polyunsaturated, preferably C₆ to C₂₆ fatty acids according to Appendix 1.
25. 9. Enzyme component system according to Claim 8, characterized in that it contains as system component 2) preferably tetradecanoic acid (myristic acid) and/or dodecanoic acid (lauric acid).
30. 10. Enzyme component system according to Claims 1 through 9, characterized in that it contains as system component 3) at least one oxidant precursor such as peroxide (H₂O₂), an organic peroxide such as 3-chloroperoxybenzoic acid, Mg monoperoxyphthalate, ditert.butyl peroxide, cumene hydroperoxide, lauroyl peroxide, chloroperoxybenzoic acid, dicumyl hydroperoxide, methyl ethyl ketone peroxide, benzoyl peroxide, diperoxydodecanedioic acid Na salt and others and a per-compound

such as a perborate, persulfate, percarbonate, perphosphate, percarbamide, perchlorate etc..

11. Enzyme component system according to Claims 1 and 10, characterized in that it contains as system component 3) the H₂O₂-activating ions Mo⁶⁺, W⁶⁺, Va⁵⁺ and/or

5 compounds such as the nitrilamines and/or dicyandiamines.

12. Enzyme component system according to Claims 1, 10 and 11, characterized in that it preferably contains H₂O₂, as system component 3).

10 13. Enzyme component system according to Claims 1, 10 and 11, characterized in that it contains as system component 3) H₂O₂ generated in situ from glucose and GOD.

14. Enzyme component system according to Claims 10 through 13, characterized in that it contains as system component 3) besides per acids also a bleaching activator such

15 as TAED (tetra- acetyl ethylenediamine), TAGU (tetraacetylglycoluril) and iso-NOBS (sodium p-Isononanoyloxy- benzenesulfonate).

15. Enzyme component system according to Claims 1 and 10 through 14,

characterized in that it contains as system component 3) besides the peroxides and/or

20 per-compounds also air or oxygen at atmospheric pressure at a slightly positive pressure of up to 2 bar.

16. Enzyme component system according to Claims 1 through 15, characterized in that it contains as system component 4) at least one ketone of general formula I:

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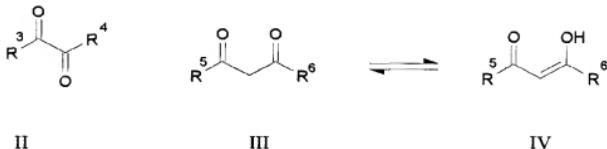
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The R^1 and R^2 groups can be equal or different and denote aliphatic or aromatic groups. Moreover, the R^1 and R^2 groups can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen and sulfur.

5 17. Enzyme component system according to Claims 1 through 16, characterized in
that it contains as system component 4) a 1,2-diketone of formula II, a 1,3-diketone of
formula III or a polyketone (polyketide) as well as a tautomeric enol of formula IV

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wherein the R^3 to R^6 groups, once again, can be equal or different and denote aliphatic or aromatic groups. Moreover, groups R^3 and R^4 and groups R^5 and R^6 , together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur.

18. Enzyme component system according to Claims 1 through 17, characterized in
that it contains as system component 4) besides a general carbonyl compound also a
ketone such as, in general, a hydroxyketone, α,β -unsaturated ketone, oxydicarboxylic
acid, quinone and halogenated ketone.

19. Enzyme component system according to Claims 1 through 18, characterized in that it contains as system component 4) a compound such as those listed in Appendix 2.

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20. Enzyme component system according to Claims 1 through 19, characterized in that it contains a polymerization catalyst such as, in particular, a phenolic substance or polycyclic compound with several oxidizable hydroxyl groups according to Appendix 3.

5 21. Enzyme component system according to Claims 1 through 20, characterized in that it is possible to add to it as an additional system an enzymatic oxidation system with enzyme action- enhancing compounds, said system containing

- a) at least one suitable oxidation catalyst
- b) at least one suitable oxidant

10 c) at least one mediator selected from the group consisting of hydroxylamines, hydroxylamine derivatives, hydroxamic acids, hydroxamic acid derivatives, aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing at least one N-hydroxy, oxime, N-oxy or N,N'-dioxy function and/or at least one mediator from the group of amides, such as, for example, hydrazides or 1,2,4-triazolidin- 3,5-diones (urazoles)

15 and/or at least one mediator from the group of imides such as, for example, the hydantoins, and/or at least one mediator from the group of oxocarbons.

22. Enzyme component system according to Claims 1 through 21, characterized in that it is possible to add to it as an additional system an enzymatic oxidation system with enzyme action- enhancing compounds, said system containing:

20 at least one mediation enhancer selected from the group consisting of carbonyl compounds, aliphatic ethers, phenol ethers or olefins (alkenes) and/or at least one mediation enhancer selected from the group consisting of NO⁻, NOH⁻ and H⁺OH⁻ compounds and/or amides such as hydrazides or urazoles and/or imides such as

25 hydantoins and/or oxocarbons.

23. Enzyme component system according to Claims 1 through 22, characterized in that it is possible to add to it as an additional system an enzymatic oxidation system with enzyme action- enhancing compounds, said system containing:

30 at least one mediation enhancer selected from the group consisting of cation radical-generating substances, of the phenothiazine and/or phenoxazine type and/or of the (R=N-N=R) type (for example, ABTS) or of aryl-substituted alcohols (nonphenols)

such as, for example, veratryl alcohol and/or phenol derivatives, such as p-hydroxycinnamic acid, 2,4-dichlorophenol, p-hydroxy- benzenesulfonate, vanillin (4-hydroxy-3-methoxybenzaldehyde), p-hydroxybenzoic acid, 5-amino-2- hydroxybenzoic acid (5-aminosalicylic acid) and/or Wurster-type radical cation compounds, such as p-phenylenediamine, preferably N,N-dimethyl-p-phenylenediamine, N,N-diethyl-p-phenylenediamine, N,N,N',N'-tetramethyl-p-phenylenediamine, 2,3,5,6-tetramethyl-p-phenylenediamine and/or radical anions, for example semiquinones, which can be generated by enzymatic oxidation of hydroquinones.

10 24. Enzyme component system according to Claims 1 through 21, characterized in that it is possible to use as oxidation catalysts enzymes such as the oxidoreductases of classes 1.1.1. to 1. 97, and preferably:
cellobiose: oxygen-l-oxidoreductase (cellobiose oxidase) (1.1.3.25),
cellobiose: quinone-l-oxidoreductase (1.1.5.1), bilirubin oxidase (1.3.3.5), cytochrome 15 oxidase (1.9.3), oxygenases, lipoxygenases (1.13, 1.14),
superoxide dismutase (1.15.11), ferrioxidase, for example ceruloplasmin (1.16.3.1),
1.10 such as catechol oxidase (tyrosinase) (1.10.3.1), L-ascorbate oxidase (1.10.3.3),
O-aminophenol oxidase (1.10.3.4) and laccase (benzodiol:oxygen oxidoreductase)
(1.10.3.2), 1.11 such as cytochrome C peroxidase (1.11.1.5), catalase (1.11.1.6),
20 peroxidase (1.11.1.7), iodide peroxidase (1.11.1.8), glutathione peroxidase (1.11.1.9),
chloride peroxidase (1.11.1.10) and L- ascorbate peroxidase (1. 11. 1. 11), phospholipid
hydroperoxide glutathione peroxidase (1. 11. 1. 12), manganese peroxidase
(1.11.1.13) and diarylpropane peroxidase (ligninase, lignin peroxidase) (1.11.1.14).

25. Enzyme component system according to Claims 1, 21 and 24, characterized in that enzymes such as laccases and/or peroxidases are preferably used as oxidation catalysts.

26. Enzyme component system according to Claim 25, characterized in that it preferably contains laccases and/or peroxidases from white rotting fungi such as, for example, *Trametes versicolor*, *Trametes* spec., *Phlebia* spec., *Pleurotus* spec.,
30 *Phanerochaete chrysosporium*, *Agaricus* spec. etc. and also other fungi, bacteria, plant

and animal cells obtained from natural organisms or organisms modified by genetic engineering.

27. Enzyme component system according to Claims 1, 21 to 26, characterized in that
5 modified enzymes, enzyme constituents, prosthetic groups or mimicking substances are used as the enzyme catalysts.

28. Enzyme component system according to Claims 21 through 27, characterized in that it employs as additional oxidants preferably air, oxygen, ozone, a peroxide such as
10 H_2O_2 , an organic peroxide, a peracid such as peracetic, performic, persulfuric, pernitric, metachloroperoxybenzoic and perchloric acid, a per-compound such as a perborate, percarbonate and persulfate, or oxygen species and the radicals thereof such as the OH^- , OOH and OH^+ radical, superoxide (O_2^-), dioxygenyl cation (O_2^+), singlet oxygen, ozonide (O_3), dioxiranes, dioxitanes or Fremy radicals.

15 29. Enzyme component system according to Claims 21 through 28, characterized in that the mediators and additional mediation enhancers are those shown in Appendix IV and IVa.

20 30. Enzyme component system according to Claims 21 through 29, characterized in that the mediator/mediation enhancer ratio is from 5000:1 to 5:1 and preferably from 500:1 to 5:1.

31. Use of the enzyme component system according to Claims 1 to 30 in a process for
25 the delignification and/or modification and/or bleaching of cellulose/wood pulp from wood or annual plants and of high yield wood pulps (groundwood and refiner pulp) and deinked pulps, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 11, preferably at pH 3 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, at a pulp consistency from 0.5 to 40%, preferably from 4 to 15%, in 30 the presence of oxygen or air at atmospheric pressure or a slightly positive pressure (up to 2 bar), and system component 1, namely lipase from *Humicula lanuginosa*, is used at

a concentration from 0.05 to 5 mg, preferably from 0.05 to 2 mg, and amidase from *Pseudomonas aeruginosa* is used at a concentration from 40 to 200 IU,

and system component 2, namely one or more fatty acids, preferably C₈ to C₁₆ fatty acids, preferably tetradecanoic and/or dodecanoic acid, are used at a concentration from

5 0.05 to 20 mg, preferably from 0.05 to 10 mg,

and system component 3, namely the oxidant precursor, preferably H₂O₂, is used at a concentration from 0.05 to 20 mg (100%), preferably from 0.05 to 10 mg,

and system component 4, namely a ketone, preferably benzophenone, is used at a concentration from 0.05 to 20 mg, preferably at a concentration from 0.05 to 10 mg, in

10 each case based on 1 g of absolutely dry pulp.

32. Use of the enzyme component system according to Claims 1 and 31 in a process for the delignification and/or modification and/or bleaching of cellulose/wood pulp from wood or annual plants and of high yield wood pulps (groundwood and refiner pulps)

15 and deinked pulps, whereby an acid wash or a Q-step is used before and/or after the reaction of the enzyme component system and the acid wash is carried out at 60-120 °C, at pH 2 to 5.5, for 30-90 min and at 4-20% pulp consistency, and the Q-step is carried out with 0.05-1 %, preferably with 0.2 to 0.5% of chelator at 60-100 °C, at pH 2 to 5.5 for 30-90 min and at a pulp consistency of 4-20%.

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33. Use of the enzyme component system according to Claims 1, 31 and 32 in a process for the delignification and/or modification and/or bleaching of cellulose/wood pulp from wood or annual plants and of high yield wood pulps (groundwood and refiner pulps) and deinked pulps, whereby the acid wash and the Q-step are carried out for 1

25 hour at 60- 90 °C, at pH 2 to 5 and at 10% pulp consistency.

34. Use of the enzyme component system according to Claims 1, 31 to 33 in a process for the delignification and/or modification and/or bleaching of cellulose/wood pulp from wood or annual plants and of high yield wood pulps (ground wood and refiner pulps)

30 and deinked pulps, whereby said system can be used before or after any possible treatment of the pulp by single or multiple digestion, bleaching steps or other pre- and post-treatments, such as alkaline leaching, alkaline extraction, washing, acid treatment,

Q-step, O₂- delignification step, peroxide bleaching step, O₂-promoted peroxide step, pressurized peroxide step, peracid step, peracid- promoted O₂ or peroxide step, ozone bleaching step, dioxirane step, polymethoxalate step, Cl₂-delignification step, ClO₂- bleaching step, Cl₂ /ClO₂- bleaching step, reductive bleaching steps, sulfonation steps,

5 NO/NO₂ treatments, nitrosylsulfuric acid treatment, swelling steps, enzyme treatments, for example treatments with hydrolases such as cellulases and/or hemicellulases (for example, xylanase, mannase, etc.) and/or pectinases and/or proteinases and/or lipases and/or amidases and/or oxidoreductases, such as, for example, laccases and/or peroxidases etc. or several combined treatments.

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35. Use of the enzyme component system according to Claims 1 and 34 in a process for the delignification and/or modification and/or bleaching of cellulose/wood pulp from wood or annual plants and high yield wood pulps (groundwood and refiner pulps) and deinked pulps, whereby the swelling step is carried out with the aid of substances such

15 as, for example, glycols, such as propylene glycol or ethylene glycol, glycol ethers such as ethylene glycol dimethyl ether etc., but also with the aid of solvents, for example, alcohols such as methanol, ethanol, butanol, amyl alcohol, cyclohexanol, benzyl alcohol and chlorohydrin, phenols such as phenol, methylphenols and methoxyphenols, aldehydes such as formaldehyde and chloral, mercaptans such as butyl mercaptan, 20 benzyl mercaptan and thioglycolic acid, organic acids such as formic, acetic and chloroacetic acid, amines such as ammonia and hydrazine, hydrotropic solvents, for example a concentrated solution of sodium benzoate, other substances such as benzenes, pyridines, dioxane, ethyl acetate, and other basic solvents such as OH-/H₂O or OH-/alcohol etc..

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36. Use of the enzyme component system according to Claims 1 to 35 in a process for the delignification and/or modification and/or bleaching of cellulose/wood pulp from wood or annual plants and of high yield wood pulps (groundwood and refiner pulps) and deinked pulps, whereby there is added to the reaction solution a complexing agent 30 such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydroxy- ethylenediaminetriacetic acid (HEDTA),

diethylenetriaminopentamethylenephosphonic acid (DTMPA), nitrilotriacetic acid (NTA), polyphosphoric acid (PPA) or other complexing agents for iron, manganese or copper, for example diethylamine or hydroxylamine.

5 37. Use of the enzyme component system according to Claims 1 to 36 in a process for the delignification and/or modification and/or bleaching of cellulose/wood pulp from wood or annual plants and of high yield wood pulps (groundwood and refiner pulps) and deinked pulps, said process being carried out in several steps and whereby between each step is applied a washing or washing and extraction step with alkaline hydroxide 10 solution, or neither washing nor extraction takes place.

38. Use of the enzyme component system according to Claims 1 to 37 in the treatment of paper production wastewater (grinder wastewater, TMP wastewater) and of wastewater from other branches of the industry, such as wood pulp wastewater and 15 textile production wastewater, among others, whereby the reaction of the enzyme component system is carried out at pH 2 to 11, preferably 3 to 6, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure or slightly positive O₂ pressure (up to 2 bar), and system component 1, namely lipase from *Aspergillus* spec. is used at a concentration from 0.05 to 50 mg, preferably from 0.5 to 10 mg, and system component 2, namely one or more fatty acids, preferably 20 C₈ to C₁₆ fatty acids, preferably tetradecanoic acid and/or dodecanoic acid, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and system component 3, namely the oxidant precursor, preferably H₂O₂, is used at a concentration from 0.05 to 200 mg (100%), preferably at a concentration from 0.05 to 50 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and that a polymerization catalyst, preferably purpurogallin, is used at a concentration from 0.005 to 200 mg, preferably at a concentration from 0.005 to 50 mg, the concentrations in all cases being based on 1 liter of wastewater.

30 39. Use of the enzyme component system according to Claims 1 to 38 for the production of lignin solutions or gels and of the corresponding binders/adhesives, and

for the production of wood-based composites, whereby the reaction of the enzyme component system is carried out at pH 2 to 11, preferably 3 to 6, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure or slightly positive O₂ pressure (up to 2 bar), and system component 1, namely

5 lipase from *Humicola lanuginosa* is used at a concentration from 0.05 to 50 mg, preferably from 0.5 to 10 mg, and system component 2, namely one or more fatty acids, preferably C₈ to C₁₆ fatty acids, preferably tetradecanoic acid and/or dodecanoic acid, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and system component 3, namely the oxidant precursor, preferably H₂O₂, is used
10 at a concentration from 0.05 to 200 mg (100%), preferably at a concentration from 0.05 to 50 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 200 mg, preferably at a concentration from 0.05 to 50 mg, and that a polymerization catalyst, preferably purpurogallin, is used at a
15 concentration from 0.005 to 200 mg, preferably at a concentration from 0.005 to 50 mg, the concentrations in all cases being based on 1 liter of wastewater.

40. Use of the enzyme component system according to Claims 1 to 39 in a process for the enzymatic printing ink removal during the deinking of waste paper, whereby the reaction of the enzyme component system is carried out at pH 7 to 11, preferably at pH

20 7 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure or slightly positive O₂ pressure (up to 2 bar), and system component 1, namely lipase from *Humicola lanuginosa*, is used at a concentration from 5 to 500 mg, preferably from 5 to 100 mg, and system component 2, namely one or more fatty acids, preferably C₈ to C₁₆ fatty acids, preferably
25 tetradecanoic acid and/or dodecanoic acid, is used at a concentration from 5 to 2000 mg, preferably at a concentration from 5 to 500 mg, and system component 3, namely the oxidant precursor, preferably H₂O₂ is used at a concentration from 5 to 5000 mg (100%), preferably at a concentration from 5 to 1000 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 5 to 2000
30 mg, preferably at a concentration from 5 to 500 mg, and that, to change the optimum pH for the printing ink removal reaction and to affect the swelling behavior of the waste paper, a phenolic or polycyclic substance with several oxidizable hydroxyl groups,

preferably bisphenol A, is used at a concentration from 1 to 2000 mg and from 1 to 500 mg, in each case based on 1 kg of air-dried waste paper.

41. Use of the enzyme component system according to Claims 1 to 40 in a process
5 for the enzymatic printing ink removal during the deinking of waste paper, whereby a
reducing agent such as sodium bisulfite, sodium dithionite, ascorbic acid, a thiol
compound, mercapto compound or glutathione, but preferably sodium bisulfite and/or
sodium dithionite, is added at a concentration from 0.1 to 1000 mg per kg of air-dried
waste paper and preferably at a concentration from 0.1 to 200 mg per kg of waste
10 paper.

42. Use of the enzyme component system according to Claims 1 to 41 in a process
for the enzymatic printing ink removal during the deinking of waste paper, whereby, to
collect the printing ink particles and to produce foam during flotation, a commercial
15 collector, preferably of the Incopur brand, for example Incopur RSGA, is used at a
concentration from 1 to 5000 mg per kg of air-dried waste paper and preferably from 1
to 1000 mg per kg of waste paper.

43. Use of the enzyme component system according to Claims 1 to 42 in a process
20 for the enzymatic printing ink removal during the deinking of waste paper, whereby
additional enzymes such as cellulases and/or hemicellulases such as xylanase and/or
mannase and/or pectinases and/or oxidoreductases are added.

44. Use of the enzyme component system according to Claims 1 to 43 as an
25 oxidation system in organic synthesis, whereby the reaction of the enzyme component
system is carried out at pH 2 to 11, preferably pH 3 to 6, at a temperature from 20 to 95
°C, preferably from 40 to 95 °C, in the presence of oxygen or air at atmospheric pressure
or slightly positive O₂ pressure (up to 2 bar), and system component 1, namely lipase
from *Humicola lanuginosa* is used at a concentration from 0.05 to 5 mg, preferably from
30 0.05 to 3 mg, and system component 2, namely one or more fatty acids, preferably C₈ to
C₁₆, fatty acids, preferably tetradecanoic acid and/or dodecanoic acid, is used at a
concentration from 0.05 to 100 mg, preferably at a concentration from 0.05 to 30 mg.

and system component 3, namely the oxidant precursor, preferably H_2O_2 is used at a concentration from 0.05 to 100 mg (100%), preferably at a concentration from 0.05 to 30 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 100 mg, preferably at a concentration from 0.05 to 30 mg, the concentrations in all cases being based on 10 mmoles of substrate.

5 45. Use of the enzyme component system according to Claims 1 to 44 as an oxidation system in organic synthesis, whereby, for example, an aromatic alcohol or an aromatic methyl compound is used as the substrate for the oxidation reaction according to the invention.

10 46. Use of the enzyme component system according to Claims 1 to 45 in a process for the enzymatic liquefaction of coal, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 11, preferably at pH 3 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, at a coal slurry consistency from 0.5 to 40%, preferably from 4 to 15%, in the presence of oxygen or air at atmospheric pressure or a slightly positive O_2 pressure (up to 2 bar), and system component 1, namely lipase from *Humicula lanuginosa* is used at a concentration from 0.05 to 20 mg, preferably from 0.05 to 10 mg, and system component 2, namely one or more fatty acids, preferably C_8 to C_{16} fatty acids, preferably tetradecanoic and/or dodecanoic acid, is used at a concentration from 0.05 to 100 mg, preferably from 0.05 to 50 mg, and system component 3, namely the oxidant precursor, preferably H_2O_2 , is used at a concentration from 0.05 to 50 mg (100%), preferably from 0.05 to 50 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 100 mg, preferably at a concentration from 0.05 to 50 mg, in each case based on 1 g of coal (lignite).

15 47. Use of the enzyme component system according to Claims 1 to 46 in a process for detergent bleaching, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 12, preferably at pH 3 to 10, at a temperature from 20 to 95 °C, preferably from 30 to 95 °C, in the presence of oxygen or air at atmospheric pressure or a slightly positive O_2 pressure (up to 2 bar), and system component 1, namely lipase

from *Humicula lanuginosa*, is used at a concentration from 0.05 to 20 mg, preferably from 0.05 to 10 mg, and system component 2, namely one or more fatty acids, preferably C₈ to C₁₆ fatty acids, preferably tetradecanoic and/or dodecanoic acid, is used at a concentration from 0.05 to 50 mg, preferably at a concentration from 0.05 to 20 mg.

5 and system component 3, namely the oxidant precursor, preferably H₂O₂, is used at a concentration from 0.05 to 50 mg (100%), preferably at a concentration from 0.05 to 20 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 50 mg, preferably at a concentration from 0.05 to 20 mg, in each case based on 100 mL of washing solution.

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48. Use of the enzyme component system according to Claims 1 to 47 in a process for detergent bleaching, whereby the system is added to a detergent formulation with all its technically common and known detergents or detergent additives.

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49. Use of the enzyme component system according to Claims 1 to 48 in a process for bleaching and/or decolorizing textile fabrics, whereby the reaction of the enzyme component system is carried out at a pH from 2 to 11, preferably at pH 3 to 9, at a temperature from 20 to 95 °C, preferably from 40 to 95 °C, at a fabric density from 0.5 to 40% and preferably from 4 to 15%, in the presence of oxygen or air at atmospheric pressure or at a slightly positive O₂ pressure (up to 2 bar), and system component 1, namely lipase from *Humicula lanuginosa* is used at a concentration from 0.05 to 10 mg, preferably from 0.05 to 5 mg, and system component 2, namely one or more fatty acids, preferably C₈ to C₁₆ fatty acids, preferably tetradecanoic and/or dodecanoic acid, is used at a concentration from 0.05 to 20 mg, preferably from 0.05 to 10 mg, and system component 3, namely the oxidant precursor, preferably H₂O₂, is used at a concentration from 0.05 to 20 mg (100%), preferably at a concentration from 0.05 to 10 mg, and system component 4, namely a ketone, preferably a benzophenone, is used at a concentration from 0.05 to 20 mg, preferably at a concentration from 0.05 to 10 mg, in each case based on 1 g of denim.

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WO98/59108

PCT/DE 98/01689

**OXIDATION AND BLEACHING SYSTEM WITH ENZYMATICALLY
5 PRODUCED OXIDIZING AGENTS**

It is known from a number of literature references and review articles, for example from "Preparative Biotransformations" by S.M. Roberts, K. Wiggins and G. Casy, J. Wiley & Sons Ltd, that enzymes such as certain lipases are capable of forming epoxides via the formation of peroxy acids (perfatty acids). For example, in the lipase system (from 10 *Candida antarctica*), with continuous addition of H₂O₂ and in the presence of certain fatty acids, for example tetradecanoic (myristic) acid or dodecanoic (lauric) acid, from cyclooctene the corresponding epoxide is produced. It is also known that manganese peroxidases + unsaturated fatty acids can generate peracids which in turn can act as a 15 H₂O₂ source for manganese peroxidases (literature: B.W. Bogan et al., Applied and Environmental Microbiology, vol. 62, No. 5, pp. 1788-1792).

There are also a few patents that describe the formation of peracids with the aid of 20 haloperoxidases.

It is also known that dimethyldioxirane can be generated in situ from peracids or salts of peracids (such as Oxone) and acetone as the simplest ketone. Ketones other than acetone are also used. Dioxiranes can also be prepared as pure substances before they are used as oxidants, but their stability is problematical (WO 92/13993).

25 Moreover, it is known from Canadian Patent 1 129 162, US 5, 034 096 and WO 96/13634 that certain metal ions, for example Mo⁶⁺ + H₂O₂ and nitrilamides + H₂O₂ and dicyandiamides + H₂O₂ are capable of generating dioxiranes chemically from H₂O₂. In this case, surprisingly, certain combinations are possible with the enzyme components system (ECS) of the present invention (see below), namely the enzymatic 30 generation of activated oxygen species e.g. dioxiranes can be further enhanced.

These very strong and highly selective oxidants can be used in many oxidation reactions (for example epoxide reactions etc). Recently, it has been proposed to use them, in particular, as bleaching agents in the cellulose /pulp industry. Because of the dangerous

preparation and high cost, this proposal has not found acceptance (WO 92/13993).

The object of the present invention is to provide a highly selective oxidation or bleaching system for use in cellulose /pulp bleaching or high yield wood pulp bleaching,

5 in the oxidative treatment of wastewater of all kinds, in the preparation of wood-based composites, as an enzymatic deinking system, as an oxidant in organic synthesis, in coal liquefaction, as bleaching system in detergents and as a bleaching agent or oxidant in the textile industry (for example in stone washing and fabric bleaching). Said system does not present many of the drawbacks of purely chemical systems (for example 10 environmental pollution problems) or of enzymatic systems (which often show inadequate performance and are very costly).

Surprisingly, we have now found, that an oxidation system which contains certain lipases, oxidants such as H_2O_2 , certain fatty acids and certain ketones resulted, for example, in bleaching of cellulose/pulp while at the same time the kappa number

15 (delignification) was markedly reduced, i.e. it could be clearly demonstrated, surprisingly, that when the appropriate optimal components were present in an optimum proportion and concentration relative to each other, it was possible to achieve in the aforesaid cellulose/pulp bleaching a bleaching action comparable to that of dioxirane-forming chemical systems.

20 Surprisingly, it was also possible to demonstrate considerable bleaching action in the bleaching of high yield wood pulp, in the bleaching of pulps after a deinking processes, in oxidative polymerization of lignin and/or lignin-like substances and in the oxidative treatment of wastewater of all kinds, such as wastewater from high yield wood pulp preparation (groundwood, refiner pulp), from the cellulose/pulp industry and dye-contaminated wastewater, for example from the textile industry. For most of these 25 wastewaters, besides the decolorization and oxidation and thus the "destruction" of environmental pollutants, the polymerization of lignins is the preferred application, because it causes a marked increase in molecular size and permits an easier and substantially less expensive precipitation of these polymers and thus their elimination 30 from COD considerations.

Surprisingly, we were also able to demonstrate this oxidative polymerization of lignin and/or lignin-like substances in the preparation of wood-based composites (binder

and/or adhesive preparation) by oxidative polymerization of the polyphenylpropanes present. Moreover, surprisingly, we were able to demonstrate removal of printing inks in the deinking process (probably occurring by swelling of the lignin-containing waste paper fibers). Surprisingly, we were also able to observe coal liquefaction properties in the treatment of lignite or anthracite. Moreover, also surprisingly, we found a marked and selective oxidation power in the use as "oxidant" in organic synthesis, high bleaching power when used as bleaching additive to detergents, in the general bleaching of textile fabrics and as special bleach when used in the stone washing processes, namely as a replacement for mechanical color removal and/or as postbleach in these processes. It seems possible, that the responsible oxidants(s), for example, generated from the present ketones and peracids are dioxiranes which in the above-indicated applications serve as oxidants or bleaching agents either alone or in combination with the peracids formed.

The foregoing objective is reached also by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ – C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

Description of Various Applications of the Enzyme Component System (ECS) of the Invention

I) Use in the bleaching of cellulose/ wood pulp.

II) Use:

- a) in the treatment of, primarily, wood pulp wastewater in the pulp and paper industries, and**
- b) of wastewater in other industries.**

- III) Use in the preparation of lignin solutions or gels, of the corresponding binders/adhesives and of wood-based composites.**
- IV) Use as enzymatic deinking system.**
- 5 **V) Use as oxidation systems in organic synthesis.**
- VI) Use in coal liquefaction.**
- VII) Use as bleaching agent in detergents.**
- VIII) Use in the bleaching/decolorization of textile fabrics.**

10 **I) Use of the Enzyme Component System (ECS) of the Invention in the Bleaching of Cellulose/Wood Pulp**

Wood pulp is currently produced mainly by the sulfate and sulfite processes. By both processes, pulp is made by cooking at high temperature and under pressure. The sulfate process involves the addition of NaOH and Na₂S, whereas the sulfite process uses Ca(HSO₃)₂ + SO₂, although the sodium and ammonium hydrogen sulfite salts are currently used because of their higher solubility.

15 The main objective of all processes is the removal of lignin from the plant material, wood or annual plants used.

The lignin, which together with the cellulose and hemicellulose forms the main constituent of the plant material (stalks and stems), must be removed, because it is 20 otherwise not possible to produce nonyellowing, mechanically highly resistant papers.

The processes for making high yield wood pulp involve the use of stone grinders (groundwood) or of refiners (TMP = thermomechanical pulp) which after an appropriate pretreatment (chemical, thermal or thermechemical) defibrillate the wood by milling.

25 These wood pulps still contain most of the lignin. They are used primarily for the production of newspapers, magazines etc.

The possibilities of using enzymes for lignin degradation have been under investigation for several years. The mechanism of action of such lignolytic systems was discovered

only a few years ago, when it became possible to obtain sufficient amounts of enzymes from the white rotting fungus *Phanerochaete chrysosporium* by use of proper culturing conditions and the addition of inducers. This is how the hitherto unknown lignin peroxidases and manganese peroxidases were detected. Because *Phanerochaete chrysosporium* is a very effective lignin degrader, attempts have been made to isolate its enzymes and use them in purified form for lignin degradation. This was unsuccessful, however, because it was found that the enzymes primarily cause repolymerization of lignin and not its degradation.

The same is true for other lignolytic enzyme species, such as the laccases which degrade lignin oxidatively with the aid of oxygen rather than hydrogen peroxide. It was found 10 that similar processes are at work in all cases, namely that radicals are formed which then react with each other causing the mentioned polymerization.

Currently, there are only processes based on the use of in-vivo systems (fungal systems). Optimization attempts were directed mainly toward **biopulping and 15 biobleaching.**

By biopulping is meant the treatment of wood chips with live fungal systems. There are two kinds of application forms:

- 1 . Pretreating the wood chips before charging them to the refiners or milling for the purpose of saving energy in high yield wood pulp production (for example, TMP or 20 groundwood). Another advantage is the usually achieved improvement of mechanical properties of the stock, and a drawback is that the final brightness is worse.
2. Pretreating the wood chips (softwood/hardwood) before wood pulp cooking (kraft process, sulfite process). Here, the objective is to reduce the amount of digestion chemicals, to improve digestion capacity and extended cooking. The advantages include 25 improved kappa number reduction following digestion compared to digestion without pretreatment.

The drawbacks of these processes are clearly their long treatment times (several weeks) and particularly the unsolved problem of risk of contamination during the treatment, if it is desired to omit the uneconomical sterilization of the wood chips.

Biobleaching also uses in-vivo systems. Before bleaching, the digested pulp (softwood/hardwood) is inoculated with the fungus and treated for a period of days or weeks. Only after such a long treatment time is it possible to observe a drop in kappa number and a significant improvement in brightness, so that the process is 5 uneconomical for implementation in current bleaching sequences.

Another application, mostly carried out with immobilized fungal systems, is the treatment of pulp production wastewaters, particularly bleaching plant wastewaters, for the purpose of decolorizing them and reducing the AOX value (reducing the amount of chlorinated compounds in the wastewater, compounds which were used for chlorine or 10 chlorine dioxide bleaching). It is also known to use hemicellulases and particularly xylanases and mannases as bleach boosters.

These enzymes act mainly on the reprecipitated xylan, which after the cooking process partly covers the residual lignin, for the purpose of degrading it and thus improving accessibility to the lignin of the bleaching chemicals (primarily chlorine dioxide) used 15 in the subsequent bleaching sequences. The savings in bleaching chemicals demonstrated in the laboratory have been confirmed on a large scale only to a limited extent so that this type of enzyme must also be classified as a bleaching additive.

Patent application PCT/EP 87/00635 describes a system for removing lignin from lignin-cellulose- containing material with simultaneous bleaching. The system is based 20 on the use of lignolytic enzymes from white rotting fungi with the addition of reducing agents, oxidants and phenolic compounds as mediators.

According to DE 4 008 893 C2, in addition to the redox system, mimicking substances are added which simulate the active center (prosthetic group) of lignolytic enzymes. In this manner, a marked improvement in performance is achieved.

25 According to patent application PCT/EP 92/01086, additional improvement is achieved by use of a redox cascade with the aid of phenolic or nonphenolic aromatics "balanced" in terms of their oxidation potential.

All three processes are limited in regard to their applicability on an industrial scale in that they must be used at low wood pulp consistency (up to a maximum of 4%) and, in the case of the last two applications also by the risk of leaching out metals when chelating agents are used, the metals possibly causing peroxide decomposition in the subsequent peroxide bleaching stages.

5 WO 94/12619, WO 94/12620 and WO 94/12621 disclose processes in which the peroxidase activity is increased with enhancers. Enhancers are characterized in WO 94/12619 in terms of their half-life.

According to WO 94/12620, enhancers are characterized by the formula A = N-N = B

10 where N means nitrogen and A and B are defined cyclic groups. According to WO 94/12620, enhancers are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups.

All three patent applications concern dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions used in the 15 detergent sector.

Although the applicability to lignin is mentioned in the specification of the applications, our own tests with the substances actually disclosed in these applications have shown that the claimed mediators are ineffective in increasing the bleaching action of peroxidases in the treatment of lignin-containing materials!

20 WO 94/29510 and WO 96/18770 describe a process for enzymatic delignification whereby the enzymes are used together with mediators. The mediators disclosed are, in general, compounds characterized by the structure NO-, NOH- or NRNOH.

Among the mediators disclosed in WO 94/29510 and WO 96/18770, 1-hydroxy-1H-benzotriazole (HOBT) gave the best delignification results. HOBT, however, has

25 several drawbacks:

- it is available only at a relative high price and in insufficient quantities,

- under delignification conditions, it reacts forming 1 H-benzotriazole and other colored products, this compound shows relatively low degradability and could, in large amounts, present a pollution problem,
- to a certain degree, it harms the enzymes,

5 • its delignification velocity is not very high.

Other mediator of the described NO-, NOH- and HRN-OH type do not show most of these drawbacks, but still have the disadvantage that a relatively large amount of chemicals must be used and, particularly, that because of their physiological reactivity they may not be entirely harmless (mostly because of NO- radical formation).

10 It is therefore desirable to provide systems for modifying, degrading or bleaching lignin, lignin- containing materials or similar substances, which do not have the said drawbacks or present them only to a minor degree.

Quite surprisingly, we have now found that when the enzyme component system (ECS) of the invention is used, similar or better delignification and bleaching results are
15 achieved compared to the abovesaid oxidoreductase-mediator systems, and the said drawbacks are negligible.

In other words, according the invention, the foregoing objective is reached by providing
20 an enzyme component system (**ECS**) according which contains one or more lipases,
preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases,
preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one
or more fatty acids present (preferably C₆–C₂₆ and particularly C₈ – C₁₆ fatty acids)
(**system component 2**) and in the presence of an oxidant such as a peroxide, preferably
H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as
additional component (**system component 4**), for example, dioxiranes.

IIa) Use of the Enzyme Component System (ECS) of the Invention in the Enzymatic Treatment of Special Wastewaters (Paper Industry Wastewaters from, for Example, Groundwood Plants or Refiner Plants)

Unlike most enzymes, oxidases and peroxidases exhibit low substrate specificity,

5 namely they can convert a wide range of substances, usually of phenolic nature. Without mediators, oxidases as well as many peroxidases show the tendency to polymerize phenolic substances via free radical-induced polymerization, a property which is attributed, for example, to laccase, belonging to the group of oxidases, also in nature. The ability to polymerize appropriate substances, for example lignins, namely to
10 increase the size of the molecules involved by "coupling reactions", can be utilized, for example, for the treatment of lignin- containing wastewaters in the paper industry such as TMP wastewater (wastewater from the production of thermomechanical pulp by means of refiners) and of grinder wastewater from mechanical wood pulping units.

The water-soluble lignin compounds (polyphenolpropanes) contained in these

15 wastewaters are mainly responsible for the high COD (chemical oxygen demand) and cannot be removed by conventional technology. In the water treatment plant and in the downstream waters, they are not degraded at all or they are degraded only very slowly. At very high concentrations, these compounds can even inhibit the bacteria in a water treatment plant and thus create problems.

20 In this application, the enzyme action can be observed immediately by a rapid

development of turbidity in the wastewater being treated, caused by an enlargement and thus insolubilization of lignin molecules. The target molecules (polymerized lignin) thus enlarged in molecular weight by enzymatic catalysis can be removed by appropriate treatments (by flocculation, by precipitation with, for example, aluminum

25 sulfate/sodium aluminate, optionally in the presence of cationic or anionic polyelectrolytes or by sedimentation). The wastewater then shows a markedly reduced COD. Upon disposal, such wastewater causes less pollution, namely it increases the certainty of remaining below the permissible COD limits. Thus is particularly important for not infrequently used "procedures" run at the limit.

For this treatment, for example with laccase, the cost of removing the reaction products of the enzymatic treatment by flocculation, sedimentation or precipitation or a combination of several such methods constitutes by far the predominant part of the overall cost of the process.

5 We have now found, quite surprisingly, that when the enzyme component system (ECS) of the invention is used by employing a special combination of the components, much higher efficiency than be attained than with the above-described enzymatic systems. This means that the process according to the invention represents a substantially improved system compared to the aforesaid systems employing oxidoreductases (such 10 as, for example, laccases) as oxidation catalysts, the advantages of which are, in particular, its higher oxidation power and the use of readily degradable fatty acids and ketones (for example, benzophenones) which, although raising the COD temporarily, are readily removed in the subsequent steps carried out in the water treatment plant.

In other words, according the invention, the foregoing objective is reached by providing 15 an enzyme component system (**ECS**) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) 20 (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

To this system are added other special compounds (**polymerization catalysts**) which serve as condensation nuclei and can substantially enhance lignin polymerization so that the main objective of this enzymatic wastewater treatment, which is to use the lowest 25 possible amount of the cost-intensive precipitant, can be attained.

IIb) Use in the Enzymatic Treatment of Other Industrial Wastewaters

All other industrial wastewaters containing phenolic or, in general, oxidizable substances (for example, lignin, dyes etc) can, in principle, be treated with, for example, the aforesaid oxidoreductases. Such treatment can be applied to wastewaters from

grape presses, olive presses, dyeing plants in the textile industry, wastewaters from pulping plants etc. If at all possible, to attain maximum efficiency, polluted streams should be treated before they are combined with other wastewaters.

In this case, too, we found, surprisingly, that the use of the enzyme component system

5 (ECS) of the invention is very well suited for the treatment of the abovesaid wastewaters and in some cases shows performance advantages over the oxidoreductase systems. Here, too, the abovesaid special compounds, namely **polymerization catalysts**, are used. These substances consist of phenols, phenol derivatives or other polycyclic phenolic compounds with a number of oxidizable hydroxyl groups.

10 Preferably, such **polymerization catalysts** are, for example:

alizarin, 5-amino-2-hydroxybenzoic acid, 3-aminophenol, pyrocatechol, 2,2-bis(4-hydroxyphenyl)- propane, bis(4-hydroxyphenyl)methane, quinalizarin, 4-chloro-1-naphthol, coniferyl alcohol, 2,4-di- aminophenol dihydrochloride, 3,5-dichloro-4-

15 hydroxyaniline, 1,4-dihydroxyanthraquinone, 2,2-di- hydroxybiphenyl, 4,4-dihydroxybiphenyl, 2,3-dihydroxynaphthalene, 2,6-diisopropylphenol, 3,5-di- methoxy-4-hydroxybenzhydrazine, 2,5-ditert.butylhydroquinone, 2,6-ditert.butyl-4-

methylphenol, 4-hydroxybiphenyl, 2-hydroxydiphenylmethane, 2-(2-hydroxyphenyl)benzothiazole, 5-indanol, 2-iso- propoxyphenol, 4-isopropyl-3-

20 methylphenol, 5-isopropyl-2-methylphenol, 4-isopropylphenol, lauryl gallate, 2-naphthol, 4-nonylphenol, 3-(pentadecyl)phenol, 2-propylphenol, 4-propylphenol, purpurine, pyrogallol, 4-(1,1,3,3-tetramethylbutyl)phenol, 1,2,4-trihydroxybenzene, 2,4,6-trimethylphenol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol, 3,4,5-trimethylphenol, 6,7-dihydroxy-4-methyl coumarin, 2-(2-hydroxyethoxy)benzaldehyde,

25 1 -naphthol, nordihydroguaiaretic acid, octyl gallate, silibinin, 3,4,6-trihydroxybenzoate-octylester, 2,4,6-tritert.butylphenol, 2,4-ditert.butylphenol, 2,6-dichlorophenol, indophenol, ethoxyquin, 1-aminoanthraquinone, 2-amino-5-chlorobenzophenone, 4-aminodi- phenylamine, 7-amino-4-hydroxy-2-naphthalenesulfonic acid, 2-(4-aminophenyl)-6-methylbenzothiazole, benzanthrone, trioctyl trimellitate, trans-chalcone, bis(4-aminophenyl)amine sulfate, 2,2'- ethylidenebis (4,6-ditert.butylphenol), 2,2-bis(2,6-dibromo-4-(2-hydroxyethoxyphenyl)propane, bis(3,5-ditert.butyl-4-

hydroxyphenyl)methane, 2,2-bis(3,5-dichloro-4-hydroxyphenyl)propane, Bismarck Brown Y, 1-bromophthalein, 4-butylaniline, 2-tert.butyl-5-methylphenol, 1-chloro-anthraquinone, 2-chloroanthraquinone, triallyl 1,3,5-benzenetricarboxylate, 1,1,1-tris(hydroxymethyl)propane, tri-methacrylate, pentaerythritol triacrylate, 1,2,4-

5 trivinylcyclohexane, trans,cis-cyclododeca-1 5,9-triene, pentaerythritol tetrabenoate, 4,4'-methylenebis(2,6-ditert.butylphenol), 4,4'-isopropylidene-bis(2,6-dichlorophenol), 4,4'-isopropylidene-bis(2,6-dibromophenol), 4,4'-isopropylidene-bis[2-(2,6-dibromophenoxy)ethanol, 2,2'-ethylidene-bis(4,6-ditert.butylphenol), 3-tert.butyl-4-hydroxy-5-methylphenol, 5-tert.butyl-4-hydroxy-2-methylphenol, syringaldazine, 4,4'-dimethoxytriphenylmethane and di-sec.butylphenol.

10

Also particularly preferred are compounds with several hydroxyl groups, such as:

ellagic acid, gallic acid, gallein, gallangin, myoinositol, morin, nitraniic acid,

15 phenolphthalein, purpurin, purpurogallin, quinizarin, chrysazin, quercitin, quinhydrone, chloranilic acid, carmine, rhodizonic acid, croconic acid, meilitic acid, hematoxylin, 9-phenyl-2,3,7-trihydroxy-6-fluorene, 9-methyl-2,3,7-trihydroxy-6-fluorene, tetrahydroxy-p-benzoquinone, 2,2',4,4'-tetrahydroxybenzophenone, Pyragallol Red, 1-nitrophloroglucinol, 1,4-dihydroxyanthraquinone, 5,8-dihydroxy-1,4-naphthoquinone,

20 hexa-oxocyclohexane octahydrate, 5,7-dihydroxyflavanone, 3',4'-dihydroxyflavanone, glyoxal hydrate, 1,3,5-tris(2-hydroxyethyl)isocyanuric acid, quinalizarin and 2,4,5-trihydroxybenzamine.

III) Use of the Enzyme Component System of the Invention in the Preparation of

25 **Lignin Solutions or Gels, of the Corresponding Binders/Adhesives and of Wood-Based Composites**

The object of the present invention is to provide a process for enzymatic polymerization and/or modification of lignin or lignin-containing materials, for example for use in the 30 production of wood compositions or wood-based composites such as, for example, fiber board from disintegrated wood or particle board from wood chips or wood pieces (chipboard, plywood, wood composite beams).

It is known from the literature and patents, for example WO 94/01488, WO 93/23477, WO 93/25622 and DE 3 037 992 C2 that laccases, lignin peroxidases or peroxidases can be used for this purpose. The main drawbacks, particularly in the case of laccases and lignin peroxidases, are the difficulty of preparing these enzymes and the low yields even of genetically modified systems.

We have now found, quite surprisingly, that here, too, the enzyme component system (ECS) of the invention shows much better performance compared to the prior-art enzymatic systems for the polymerization and/or modification of lignin and/or lignin-containing materials.

In other words, according the invention, the foregoing objective is reached by providing an enzyme component system (**ECS**) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

To this end, the enzyme component system of the invention is brought together with lignin (for example, with lignosulfates and/or unevaporated or evaporated sulfite waste liquor and/or sulfate lignin --> kraft lignin, for example indulin) and/or with lignin-containing material. The lignin and/or the lignin-containing material can either be preincubated at an elevated pH, namely above pH 8 and preferably at a pH between 9.5 and 10.5, at 20 to 100 °C (preferably at 60 to 100 °C) after which the pH is reduced to below pH 7, depending on the optimum pH range for enzyme activity of the component system (ECS) or, if the activity optimum of the enzyme component system (ECS) is on the alkaline side, the ECS and the lignin and/or the lignin-containing material are brought together immediately, without pretreatment. The purpose of the pretreatment or treatment under alkaline pH conditions is to utilize the substantially easier solubilization of lignin at these higher pH values. This is a major advantage for the use according to the invention, because it is thus possible to work without an organic solvent.

In other words, the main purpose of the described bringing together of the enzyme component system and the lignin and/or lignin-containing material is to achieve activation of the substrates (polyphenylpropanes) by oxidation, namely to convert the lignin and/or the lignin-containing material by free radical-induced polymerization

5 (modification) into an activated and active binder which then when brought together with the wood fibers and/or wood parts to be bonded (cemented together) can be cured under the action of pressure and elevated temperature to give solid wood-based composites such as the abovesaid wood products, for example fiber boards and particle boards. The main advantage consists of reducing, or producing savings in, the amount 10 of urea-formaldehyde resins normally used, for example, for gluing in the production of chipboard, which resins, besides being toxic, have only limited moisture resistance, or of phenol-formaldehyde resins which exhibit unfavorable swelling properties and require long pressing times (once again, besides the question of toxicity).

The polymerizing and/or modifying action of the enzyme component system can be

15 additionally enhanced by addition of certain chemical polymerization catalysts, for example polydiphenylmethane diisocyanate (PMDI) and other polymerization catalysts used also for the polymerization of lignin in lignin-containing wastewaters. Such substances consist of phenols, phenol derivatives or other polycyclic phenolic compounds with a number of oxidizable hydroxyl groups, as already indicated 20 hereinabove (wastewater treatment).

IV) Use of the Enzyme Component System (ECS) as an Enzymatic Deinking System

In principle, by deinking, which is currently always run in a conventional manner by

25 flotation, is meant a two-step process.

Its objective is to remove printing ink and other dye particles from the waste paper. The waste paper used in most cases is paper collected domestically and consists mainly of newspapers and magazines.

In the first treatment step, the dye particles adhering to the paper fibers are removed primarily by mechanical/chemical means. This is accomplished by "recycling" the paper as a uniform fibrous slurry, namely by disintegrating (communuting) the waste paper in pulpers, drums or the like with simultaneous addition of chemicals capable of enhancing removal and preventing yellowing and thus also acting as bleaching chemicals, namely sodium hydroxide solution, fatty acid, water glass and hydrogen peroxide (H₂O₂). Here, the fatty acid acts as a fiber dye particle collector and in the second treatment step, the flotation, also as foaming agent.

After the waste paper has been disintegrated and the said chemicals have been allowed to act for a certain length of time, the flotation is carried out in special flotation vessels by injecting air. During this process, the dye particles adhere to the foam bubbles and are removed together with the bubbles. The dye is thus separated from the paper fibers. Currently, this operation is preferably carried out at a neutral pH, which makes it necessary to use certain detergents in place of the fatty acids.

It is known from the literature (WO 91/14820, WO 92/20857) to use an oxidoreductase or laccase system characterized primarily by the addition of special substances which cause the optimum pH for the action of laccase obtained from *Trametes versicolor*, which normally is in the range of about pH 4-5, to shift into the slightly alkaline range (pH 8 to 8.7). This, on the one hand, is an important prerequisite for use in the deinking system because of the CaSO₄ problems arising below pH 7 and, on the other, does not optimize the action of laccase in the polymerizing or depolymerizing sense, but only produces a certain swelling of the fibers. Such swelling (which is one of the main actions of sodium hydroxide in purely chemical deinking systems) is a primary performance characteristic of the dye-removing mechanism.

The only other additives required for this enzymatic system employing oxidoreductases are the detergents needed to produce foam. Nearly all suitable detergents also exert a dye-removing action. Moreover, in conventional deinking systems the use of sodium hydroxide and peroxide improves brightness as a result of the bleaching action of these chemicals. This bleaching action cannot be achieved with the prior-art enzyme system because of the nature of the system.

We have now found, quite surprisingly, that by appropriate selection of the components the enzyme component system (ECS) of the invention exceeds the efficiency of other enzymatic deinking systems, particularly those with oxidoreductases and those applied to lignin-containing deinked pulp and at least in part compensates for the advantage of 5 bleaching with purely chemical systems. This means that it is possible to provide a system offering environmentally friendly deinking under neutral pH conditions and thus better post-bleaching, better pulp properties etc and good performance similar to that of purely chemical systems.

In other words, according the invention, the foregoing objective is reached by providing 10 an enzyme component system (**ECS**) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) 15 (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

In this case, a further improvement of printing ink removal can be attained by the aforesaid addition of special substances mostly of a phenolic nature and, in particular, 20 containing several hydroxyl groups, which are also used as polymerization catalysts in enzymatic wastewater treatment and general polymerization reactions, as in the production of binders/adhesives from lignin or lignin-containing substances primarily 25 for the preparation of wood-based composites.

V) Use of the Enzyme Component System (ECS) of the Invention as an Oxidation System in Organic Synthesis

Recently, enzymes have increasingly been used for chemical reactions in organic synthesis. A few examples showing a variety of oxidative reactions that can be carried out with enzymatic systems can be found in: *Preparative Biotransformations (Whole Cell and Isolated Enzymes in Organic Synthesis)*, S.M. Roberts, K. Wiggins and G. 30 Casy, J. Wiley & Sons Ltd, 1992/93; *Organic Synthesis with Oxidative Systems*, H.L.

Holland, VCH, 1992; and **Biotransformations in Organic Chemistry**, K. Faber, Springer Verlag [publisher], 1992:

1) Hydroxylation reactions

- 5 a) Synthesis of alcohols
- b) Hydroxylation of steroids
- c) Hydroxylation of terpenes
- d) Hydroxylation of benzenes
- e) Hydroxylation of alkanes
- 10 f) Hydroxylation of aromatic compounds
- g) Hydroxylation of double bonds
- h) Hydroxylation of nonactivated methyl groups
- i) Dihydroxylation of aromatic compounds

15 2) Oxidation of unsaturated aliphatics

- a) Preparation of epoxides
- b) Preparation of compounds by epoxidation
- c) Preparation of arene oxides
- d) Preparation of phenols
- 20 e) Preparation of cis-dihydrodiols

3) Baeyer-Villiger oxidations

- a) Baeyer-Villiger conversion of steroids

4) Oxidation of heterocycles

- 25 a) Transformation of organic sulfides
- b) Oxidation of sulfur compounds
- c) Oxidation of nitrogen compounds (formation of N-oxides etc.)

d) Oxidation of other heteroatoms

5) Carbon-carbon dehydrogenation

a) Dehydrogenation of steroids

6) Other oxidation reactions

- 5 a) Oxidation of alcohols and aldehydes
- b) Oxidation of aromatic methyl groups to aldehydes
- c) Oxidative coupling of phenols
- c) Oxidative degradation of alkyl chains (β -oxidation etc.)
- e) Formation of peroxides or percompounds
- 10 f) Initiation of free-radical induced chain reactions.

Here, too, we found, quite surprisingly, that with the aid of the enzyme component system (ECS) of the invention it is possible to carry out many oxidation reactions exemplified hereinabove, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (system component 1) which from one or more fatty acids present (preferably C₆–C₂₆ and particularly C₈–C₁₆ fatty acids) (system component 2) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (system component 3) can produce peracids and in the presence of ketones as additional component (system component 4), for example, dioxiranes.

VI) Use of the Enzyme Component System (ECS) in Enzymatic Coal Liquefaction

In this field, the prior art is as follows:

25 Preliminary studies show that, in principle, lignite and anthracite can be attacked and liquefied by in-vivo treatment with, for example, a white rotting fungus such as *Phanerochaete chrysosporium* (incubation time: several weeks/Bioengineering 8, 4,

1992). The possible structure of anthracite is a tridimensional network of polycyclic, aromatic ring systems with a "certain" similarity to lignin structures. Assumed cofactors besides the lignolytic enzymes are chelating agents (siderophors, such as ammonium oxalate) and biosurfactants.

- 5 1) Until now, effective coal liquefaction systems are known only as in-vivo systems (with lignin- degrading organisms, particularly white rotting fungi), or as systems employing oxidoreductases plus mediators (laccase-mediator system -> WO 94/29510; WO 96/18770).
- 10 2) It has been proven that, in principle, white rotting fungi that are capable of degrading lignin in vivo can also liquefy coal in culture.
- 15 3) Coal: Both lignite and anthracite were formed from wood by chemical/physical "actions"; hence, their chemical structures are at least similar to those occurring in lignin.
- 20 4) In coal liquefaction with white rotting fungi, we see, on the one hand, an alkalization of the pH during fungal growth "on coal" and, on the other, a secretion of siderophor-like chelators, namely substances known to have a positive effect on coal liquefaction.

The main reason for economical, meaningful industrial coal liquefaction is the industrial demand for alternative liquid sources of energy, especially considering that in the future the quantities of other sources of fossil energy such as oil and gas will be decreasing

- 25 while at the same time the demand for energy will be increasing, and that other alternatives such as nuclear fusion, among others, will not yet be available.
- 30 Here, too, we found, quite surprisingly, that with the aid of the enzyme component system (ECS) of the invention liquefaction of, for example, lignite is possible with better performance than with the conventional enzymatic oxidoreductase systems, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably

from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ – C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as 5 additional component (**system component 4**), for example, dioxiranes.

VII) Use of the Enzyme Component System (ECS) as Bleaching Agent in Detergents

10 Conventional bleaching systems in domestic detergents are unsatisfactory, particularly in the low- temperature range. Below a washing temperature of 60 °C, the standard bleaching agent, i.e., H₂O₂ / sodium perborate/sodium percarbonate must be activated by the addition of chemical bleach activators, such as TAED and/or SNOBS*. Also, the need exists for more highly biodegradable, bio-compatible bleaching systems and 15 systems for low-temperature washing that can be used in small amounts. Whereas enzymes are already being used industrially for protein-starch-fat solution and for fiber treatment in the washing process, no enzymatic system is currently available for detergent bleaching. WO 1/05839 describes the use of different oxidative enzymes (oxidases and peroxidases) to prevent dye transfer. As is known, peroxidases are 20 capable of "decolorizing" different pigments (3-hydroxyflavone and betaine are decolorized by horseradish peroxidase and carotene by peroxidase). Said patent application describes the decolorization (also referred to as bleaching) of textile dyes removed from the laundered goods and present in the washing liquor (conversion of a colored substrate into a noncolored, oxidized substance). In this case, the enzyme is said 25 to have the advantage over, for example, hypochlorite which attacks dyes in or on the fabric, in that the enzyme decolorizes only the dissolved dyes. Hydrogen peroxide or an appropriate precursor generating hydrogen peroxide in situ participates in the catalysis of the decolorization. The enzyme reaction can be enhanced somewhat by addition of other oxidizable enzyme substrates, for example metal ions such as Mn⁺⁺, halide ions, 30 such as Cl⁻ or Br⁻ or organic phenols, such as p-hydroxybenzoic acid and 3,4-dichlorophenol. In this case, it is postulated that short-lived radicals or other oxidized

states of the added substrate are formed and are responsible for the bleaching or other modification of the colored substance.

US 4, 077 6768 describes the use of iron porphin, hemin chloride, iron phthalocyanines or derivatives thereof together with hydrogen peroxide for preventing dye transfer.

5 These substances, however, are rapidly destroyed by excess peroxide, and for this reason hydrogen peroxide formation must occur in a controlled fashion. Processes are known from WO 94/12619, WO 94/12620 and WO 94/12621 whereby the activity of the peroxidase is enhanced by means of enhancers. Such enhancers are characterized in WO 94/12620 in terms of their half-life. According to WO 94/12621, 10 enhancers are characterized by the formula A = N-N = B where N means nitrogen and A and B are defined cyclic groups. According to WO 94/12620, enhancers

* TAED = tetraacetylenediamine; SNOBS = sodium nonyloxybenzenesulfonate

15 are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups. All three patent applications concern dye transfer inhibition and the use of enhancers together with peroxidases as detergent additives or detergent compositions used in the detergent sector. The combination of these enhancers is limited to peroxidases. The use 20 of mixtures containing peroxidases is also known from WO 92/18687. A special system of oxidases and of appropriate substrates such as hydrogen peroxide is described in German Unexamined Patent Application DE-42 31 761. German Unexamined Patent Application DE 19 18 729 concerns another special detergent system consisting of glucose and glucose oxidase or of starch, aminoglucosidase and glucose oxidase (GOD) 25 and of added hydroxylamine or a hydroxylamine compound, wherein the hydroxylamine or the derivatives thereof serve to inhibit the catalase that is often present in GOD. Hydroxylamine and the derivatives thereof have definitely not been described as mediator additives. Finally, WO 94/29425, DE 4445088.5 and WO 97/48786 concern multicomponent 30 bleaching systems for use with detergents and which consist of oxidation catalysts and oxidants and of aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing NO-, NOH- or H-NR-OH groups.

All hitherto known "enzymatically enhanced" detergent-bleaching systems have the drawback that their cleaning and bleaching action is still unsatisfactory and that the mediators must be used in excessive amounts which may cause environmental and economic problems.

5 We have now found, quite surprisingly, that the enzyme component system (ECS) of the invention exceeds the performance of the aforesaid oxidoreductase-mediator systems and does not have the said drawbacks of the prior art, namely that the aforesaid objective can be reached by providing an enzyme component system (ECS) according to the invention which contains one or more lipases, preferably 10 from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as 15 additional component (**system component 4**), for example, dioxiranes.

VIII) Use of the Enzyme Component System of the Invention in the Bleaching and/or Decolorization of Textile Fabrics

20 Enzymes are currently being used to an increasing extent in various applications in the textile industry. For example, the use of amylases in the desizing process is of great importance, because the use of strong acids, alkalies or oxidants is thereby avoided. Similarly, cellulases are used for biopolishing and biostoning, a process which is mostly employed together with conventional stone washing with pumice in the treatment of 25 denim fabrics for jeans to remove the indigo dye. WO 94/29510, WO 96/18770, DE 196 12 194 A1 and DE 44 45 088 A1 describe enzymatic delignification processes which use enzymes together with mediators. In general, the disclosed mediators are compounds with the NO-, NOH- or HRNOH structure. These systems, of course, are restricted to use in pulp bleaching. Because the mechanisms underlying lignin-removing pulp 30 bleaching, and this is the process involved here, are entirely different from those underlying the decolorization, removal and/or "destruction" of denim dyes, particularly indigo dyes, in the jeans producing sector, it is entirely surprising that a number of

substances of the said NO-, NOH- and HNROH types are also suitable for this application.

WO 97/06244 describes systems for the bleaching of pulp, for dye transfer inhibition and for bleaching stains when used with detergents, which systems employ enzymes

5 (peroxidases, laccases) and enzyme-enhancing (hetero-)aromatic compounds, such as nitroso compounds etc.. In this case, as in patents WO 94/12619, WO 94/12620 and WO 94/12621, only the above-described use is intended. The mechanisms of stain decolorization in detergent bleaching or of dye transfer inhibition are entirely different from those underlying the decolorization, removal and/or "destruction" of indigo dyes,
10 as, for example, in denim treatment. Hence, it is quite surprising that a number of substances of the said NO-, NOH- and HNROH-types are also suitable for this application.

Processes are known from said WO 94/12619, WO 94/12620 and WO 94/12621 in which the activity of peroxidase is increased by use of enhancers. Such enhancers are
15 characterized in WO 94/12620 in terms of their half-life. According to WO 94/12621, enhancers are characterized by the formula A=N-N=B where N means nitrogen and A and B are defined cyclic groups. According to WO 94/12621, enhancers are organic chemicals containing at least two aromatic rings of which at least one is substituted with defined groups. All three applications concern (as already stated) dye transfer inhibition
20 and the use of enhancers together with peroxidases as detergent additives or detergent compositions for washing applications or in cellulose bleaching. The combinations of these enhancers are restricted to use with peroxidases.

Moreover, oxidoreductases, primarily laccases, but also peroxidases, are currently used, mainly for treating denim for jeans.

25 It is known from patent application WO 96/12846 that laccase and peroxidase + certain enhancers, mainly derivatives of phenothiazine or phenoxazine, are used in two application forms in the treatment of cellulose-containing fabrics, such as cotton, viscose, rayon (artificial silk), ramie, linen, Tencel, silk or mixtures thereof or mixtures of these fabrics with synthetic fibers, for example a mixture of cotton and
30 spandex (stretch denim), but mainly denim fabrics (mainly for use in jeans).

On the one hand, the system (oxidoreductases + enhancers) is intended as a replacement for the conventional hypochlorite bleaching of denim, usually after a stone washing pretreatment, this enzymatic treatment providing only partial replacement of hypochlorite, because the desired bleaching effect cannot be attained.

5

On the other hand, the system can be used together with cellulase in stone washing in place of the usual mechanical treatment with pumice, and this represents an improvement over the "treatment with cellulase only".

The main drawbacks of the system described in WO 96/12846 are the following, among others:

- 10 1) To achieve the desired goal, laccase must be used in considerable amounts (about 10 international units [IU]/g of denim);
- 2) In some cases, optimum treatment requires 2-3 hours;
- 3) The preferred mediator (here phenothiazine-10-propionic acid) must be used in an 15 amount of about 2 to about 14 mg per gram of denim, which represents a considerable cost;
- 4) Buffer systems (about 0.1 mol/L) must be used, because otherwise no performance can be achieved, and this also raises the cost of the system. **This, for example, is not required for the system of the invention;**
- 20 5) The color of the enhancer component (long-lived radical) causes "browning" of the fabric.

The general advantage of a laccase and/or oxidoreductase system with enzyme action-enhancing compounds (enhancers, mediators etc.) when used in the above-described treatment of textiles (for example, jeans fabrics) consists, in an improved system over the prior-art systems, in that fashion looks can be achieved, which is not possible with conventional hypochlorite bleaching.

The dyes normally used for jeans denim are vat dyes, such as indigo or indigo derivatives, for example thioindigo, as well as sulfur dyes. By use of such special enzymatic systems, it is possible (as a result of the high specificity of such systems), 30 when a mixed dye system such as an indigo dye and a sulfur dye system is present, to decolorize only the indigo dye, while the sulfur dye is not oxidized. Depending on the

enzyme action-enhancing compound used, this can produce almost any desired fabric color (for example, gray shades etc), which is often desirable.

An additional advantage is that the enzymatic treatment is substantially more gentle than bleaching with hypochlorite, and as a result fiber damage is reduced.

5 In the stone washing process, the ecological effect is of particular importance (in addition to the reduced fiber damage caused by enzymes) considering, for example, that this purely mechanical process produces about 1 kg of stone sludge per kg of jeans denim.

As can be seen from the prior art, for colored fabrics, in particular, the textile industry 10 has a great need for alternative bleaching processes (alternatives to conventional hypochlorite bleaching) and/or treatment methods as alternatives to stone washing to achieve the bleached look, in the latter case also because of the environmental pollution problems.

The present invention has for an object to minimize or eliminate the drawbacks of the 15 conventional processes: stone washing/bleaching after stone washing or general bleaching of dyed and/or undyed textile fabrics, particularly the pollution problems and fiber damage, as well as the drawbacks of the known oxidoreductase/enhancer systems (for example also NO-radical formation etc).

Entirely surprisingly, we have now found that the enzyme component System (ECS) of 20 the invention exceeds the performance of the aforesaid oxidoreductase-mediator systems and that it does not exhibit the said drawbacks of the prior art.

In other words, according the invention, the foregoing objective is reached by providing 25 an enzyme component system (**ECS**) according which contains one or more lipases, preferably from the group of triacylglycerol lipases (3.1.1.3), or one or more amidases, preferably from the group of amidases (3.5.1.4) (**system component 1**) which from one or more fatty acids present (preferably C₆- C₂₆ and particularly C₈ - C₁₆ fatty acids) (**system component 2**) and in the presence of an oxidant such as a peroxide, preferably H₂O₂ (**system component 3**) can produce peracids and in the presence of ketones as additional component (**system component 4**), for example, dioxiranes.

Description of Individual System Components of the Enzyme Component System (ECS) of the Invention

System Component 1 (Lipases and Other Enzymes

5

Preferred are enzymes of group 3 (hydrolases), 3.1, 3.1.1, 3.1.2, 3.1.3, 3.1.4 and 3.1.7 according to the International Enzyme Nomenclature: Committee of the International Union of Biochemistry and Molecular Biology (Enzyme Nomenclature, Academic Press, Inc., 1992, pp. 306-337).

10 Preferred are enzymes acting on ester bonds (3.1, particularly those acting on carboxylate esters (3.1.1):

A) Carboxylate Ester Hydrolases (3.1.1)

3.1.1.1 Carboxylate esterase

15 3.1.1.2 Aryl esterase

3.1.1.3 Triacylglycerol lipase

3.1.1.4 Phospholipase A₂

3.1.1.5 Lysophospholipase

3.1.1.6 Acetyl esterase

20 3.1.1.7 Acetylcholine esterase

3.1.1.8 Choline esterase

3.1.1.10 Tropine esterase

3.1.1.11 Pectin esterase

3.1.1.13 Sterol esterase

25 3.1.1.14 Chlorophyllase

3.1.1.15 L-Arabinolactonase

3.1.1.17 Gluconolactonase

3.1.1.19 Uronolactonase

3.1.1.20 Tannase

30 3.1.1.21 Retynil palmitate esterase

3.1.1.22 Hydroxybutyrate dimer hydrolase

3.1.1.23 Acylglycerol lipase

3.1.1.24 3-Oxoadipate enol lactonase
3.1.1.25 1,4-Lactonase
3.1.1.26 Galactolipase
3.1.1.27 4-Pyrodoxolactonase
5 3.1.1.28 Acylcarnitine hydrolase
3.1.1.30 D-Arabinone lactonase
3.1.1.31 6-Phosphogluconolactonase
3.1.1.32 Phospholipase A₁
3.1.1.32 6-Acetylglucose deacetylase
10 3.1.1.34 Lipoprotein lipase
3.1.1.35 Dihydrocoumarin hydrolase
3.1.1.36 Limonine D-ring lactonase
3.1.1.37 Steroid lactonase
3.1.1.38 Triacetate lactonase
15 3.1.1.39 Actinomycin lactonase
3.1.1.40 Orseilinate depside hydrolase
3.1.1.41 Cephalosporin C deacetylase
3.1.1.42 Chlorogenate hydrolase
3.1.1.43 α -Amino acid esterase
20 3.1.1.44 Methylxaloacetate esterase
3.1.1.45 Carboxymethylenebutenolidase
3.1.1.46 Deoxylimonate A-ring lactonase
3.1.1.47 1 -Alkyl-2-acetylglycerophosphocholine esterase
3.1.1.48 Fusarinine C ornithine esterase
25 3.1.1.49 Sinapine esterase
3.1.1.50 Wax ester hydrolase
3.1.1.51 Phorbol diester hydrolase
3.1.1.52 Phosphatidylinositol deacetylase
3.1.1.53 Sialate O-acetyl esterase
30 3.1.1.54 Acetoxybutynylbithiophene deacetylase
3.1.1.55 Acetylsalicylate deacetylase

- 3.1.1.56 Methylumbelliferyl acetate deacetylase
- 3.1.1.57 2-Pyrone-4,6-dicarboxygallate lactonase
- 3.1.1.58 N-Acetylgalactosaminoglycan deacetylase
- 3.1.1.59 Juvenile hormone esterase
- 5 3.1.1.60 Bis(2-ethylhexyl)phthalate esterase
- 3.1.1.61 Protein glutamate methylesterase
- 3.1.1.63 11-cis-Retynil palmitate hydrolase
- 3.1.1.64 all-trans-Retynil palmitate hydrolase
- 3.1.1.65 L-Rhamnono-1,4-lactonase
- 10 3.1.1.66 5-(3,4-diacetoxybutynyl)-2,2'-bitiophene deacetylase
- 3.1.1.67 Fatty acid ethyl ester synthase
- 3.1.1.68 Xylyl-1,4-lactonase
- 3.1.1.69 N-Acetylglucosaminylphosphatidylinositol deacetylase
- 3.1.1.70 Cetraxate benzyl esterase

15 Also preferred are:

B) Thiol ester hydrolases (3.1.2)

- 3.1.2.6 Hydroxylglutathione hydrolase
- 3.1.2.7 Glutathione thiol esterase
- 3.1.2.12 S-Formylglutathione hydrolase
- 20 3.1.2.13 S-Succinylglutathione hydrolase
- 3.1.2.14 Oleoyl-(acyl carrier protein) hydrolase
- 3.1.2.15 Ubiquitin thiol esterase
- 3.1.2.16 (Citrate-(pro-3S)-lyase)thiol esterase.

Also preferred are:

C) Phosphoric Acid Monoester Hydrolases (Phosphatases) (3.1.3)

- 3.1.3.1 Alkaline phosphatase
- 3.1.3.2 Acid phosphatase
- 3.1.3.3 Phosphoserine phosphatase
- 3.1.3.4 Phosphatidate phosphatase

- 3.1.3.8 3-Phytase
- 3.1.3.9 Glucose-6-phosphatase
- 3.1.3.10 Glucose-1-phosphatase
- 3.1.3.11 Fructose bisphosphatase
- 5 3.1.3.12 Trehalose phosphatase
- 3.1.3.13 Bisphosphoglycerate phosphatase
- 3.1.3.14 Methylphosphothioglycerate phosphatase
- 3.1.3.15 Histidinol phosphatase
- 3.1.3.16 Phosphoprotein phosphatase
- 10 3.1.3.17 (Phosphorylase) phosphatase
- 3.1.3.18 Phosphoglycolate phosphatase
- 3.1.3.19 Glycerol-2-phosphatase
- 3.1.3.20 Phosphoglycerate phosphatase
- 3.1.3.21 Glycerol-1-phosphatase
- 15 3.1.3.22 Mannitol-1-phosphatase
- 3.1.3.23 Sugar phosphatase
- 3.1.3.24 Sucrose phosphatase
- 3.1.3.25 Myoinositol-1 (or 4)-monophosphatase
- 3.1.3.26 6-Phytase
- 20 3.1.3.27 Phosphatidylglycerophosphatase
- 3.1.3.36 Phosphatidylinositol bisphosphatase
- 3.1.3.37 Sedoheptulose bisphosphatase
- 3.1.3.38 3-Phosphoglycerate phosphatase
- 3.1.3.39 Streptomycin-6-phosphatase
- 25 3.1.3.40 Guanidinodeoxy-scyllo-inositol-4-phosphatase
- 3.1.3.41 4-Nitrophenyl phosphatases
- 3.1.3.42 (Glycogen synthase-D) phosphatase
- 3.1.3.43 (Pyruvate dehydrogenase (lipoamide) phosphatase
- 3.1.3.44 3-Deoxy-manno-octulonante-8-phosphatase
- 30 3.1.3.46 Fructose-2,6-biphosphate-2-phosphatase
- 3.1.3.48 Protein-tyrosine phosphatase

- 3.1.3.49 (Pyruvate kinase) phosphatase
- 3.1.3.50 Sorbitol-6-phosphatase
- 3.1.3.51 Dolichyl phosphatase
- 3.1.3.52 3-Methyl-2-oxobutanate dehydrogenase) (lipoamide) phosphatase
- 5 3.1.3.53 Myosin light chain phosphatase
- 3.1.3.54 Fructose-2,6-bisphosphate-6-phosphatase
- 3.1.3.55 Caldesmon phosphatase
- 3.1.3.56 Inositol-1,4,5-triphosphate-B-phosphatase
- 3.1.3.57 Inositol-1,4-bisphosphate-l-phosphatase
- 10 3.1.3.58 Sugar terminal phosphatase
- 3.1.3.59 Alkylacetylglycerophosphatase
- 3.1.3.60 Phosphoenolpyruvate phosphatase
- 3.1.3.61 Inositol-1,4,5-trisphosphate-1-phosphatase
- 3.1.3.62 Inositol-1,3,4,5-tetrakisphosphate-3-phosphatase
- 15 3.1.3.63 2-Carboxy-D-arabinitol-1-phosphatase
- 3.1.3.64 Phosphatidylinositol-3-phosphatase
- 3.1.3.65 Inositol-1 3-bisphosphate-3-phosphatase
- 3.1.3.66 Inositol-3,4-bisphosphate-4-phosphatase.

Also preferred are:

20 **D) Phosphoric Acid Diester Hydrolases (3.1.4)**

- 3.1.4.1 Phosphodiesterase I
- 3.1.4.2 Glycerophosphocholine phosphodiesterase
- 3.1.4.3 Phospholipase C
- 3.1.4.4 Phospholpase D
- 25 3.1.4.10 1 -Phosphatidylinositol phosphodiesterase
- 3.1.4.11 1 -Phosphatidylinositol-4,5-bisphosphate phosphodiesterase
- 3.1.4.12 Sphingomyelin phosphodiesterase
- 3.1.4.13 Serine-ethanolamine phosphate phosphodiesterase
- 3.1.4.14 (Acyl carrier protein) phosphodiesterase

- 3.1.4.36 1,2-Cyclic inositol phosphate phosphodiesterase
- 3.1.4.38 Glycerophosphocholine choline phosphodiesterase
- 3.1.4.39 Alkylglycerophosphoethanolamine phosphodiesterase
- 3.1.4.40 CMP-N-acylneuraminate phosphodiesterase
- 5 3.1.4.41 Sphingomyelin phosphodiesterase D
- 3.1.4.42 Glycerol-1,2-cyclic phosphate-2-phosphodiesterase
- 3.1.4.43 Glycerophosphoinositol inositol phosphodiesterase
- 3.1.4.44 Glycerophosphoinositol glycerophosphodiesterase
- 3.1.4.45 N-acetylglucosamine-1-phosphodiesterase
- 10 3.1.4.46 Glycerophosphodiester phosphodiesterase
- 3.1.4.47 Variant surface glycoprotein phospholipase C
- 3.1.4.48 Dolochyl phosphate-glucose phosphodiesterase
- 3.1.4.49 Dolochyl phosphate-mannose phosphodiesterase
- 3.1.4.50 Glycoprotein phospholipase D
- 15 3.1.4.51 Glucose-1-phospho-D-mannosylglycoprotein phosphodiesterase.

Also preferred are:

E) Diphosphoric Acid Monoster Hydrolases (3.1.7)

- 3.1.7.1 Prenyl pyrophosphatase
- 3.1.7.3 Monoterpenyl pyrophosphatase

- 20 Particularly preferred among these are enzymes of group 3.1.1.3 lipases (triacylglycerol lipases, triglycerolacyl hydrolases) from organisms such as *Candida antarctica*, *Candida rugosa*, *Candida lipolytica*, *Candida cylindracea*, *Candida spec.*, *Geotrichum candidum*, *Humicula lanuginosa*, *Penicillium cambertii*, *Penicillium roqufortii*, *Aspergillus spec.*, *Mucor javanicus*, *Mucor mehei*, *Rhizopus arrhizus*, *Rhizopus niveus*, *Rhizopus delamar*,
- 25 *Rhizopus spec.*, *Chromobacterium viscosum*, *Pseudomonas cepacia* and *Pseudomonas spec.* from wheat seedlings or pancreas (pig or other sources) or other sources.

Enzymes splitting carbon/nitrogen (C/N) bonds (other than peptide bonds) can also be used (3.5).

This subclass includes enzymes capable of splitting amides, amidines and other C/N bonds. Particularly preferred are enzymes of class 3.5.1 which act on linear amides, of 5 class 3.5.2 which act on cyclic amides, of class 3.5.3 which act on linear amidines, of class 5.3.1 which act on nitriles and of class 3.5.99 which act on other compounds.

Particularly preferred are enzymes of class 3.5.1. which act on linear amides:

- 3.5.1.1 Asparaginase
- 3.5.1.2 Glutaminase
- 10 3.5.1.3 ω -Amidase
- 3.5.1.4 Amidase
- 3.5.1.5 Urease
- 3.5.1.6 β -Ureidopropionase
- 3.5.1.7 Ureidosuccinase
- 15 3.5.1.8 Formylaspartate deformylase
- 3.5.1.9 Arylformamidase
- 3.5.1.10 Formyltetrahydrofolate deformylase
- 3.5.1.11 Penicillin amidase
- 3.5.1.12 Biotinidase
- 20 3.5.1.13 Arylacyl amidase
- 3.5.1.14 Aminoacylase
- 3.5.1.15 Aspartoacylase
- 3.5.1.16 Acetylornithine deacetylase
- 3.5.1.17 Acyl-lysine deacetylase
- 25 3.5.1.18 Nicotinamidase
- 3.5.1.20 Citrullinase
- 3.5.1.22 Pantothenase
- 3.5.1.30 5-Aminopentanamidase
- 3.5.1.31 Formylmethionine deformylase

- 3.5.1.32 Hippurate hydrolase
- 3.5.1.39 Alkylamidase
- 3.5.1.40 Acylagmatin amidase
- 3.5.1.41 Chitin deacetylase
- 5 3.5.1.42 Nicotinamide nucleotide amidase
- 3.5.1.49 Formamidase
- 3.5.1.50 Pentanamidase
- 3.5.1.55 Long-chain fatty acylglutamate deacylase
- 3.5.1.56 N,N-Dimethylformamidase
- 10 3.5.1.57 Tryptophanamidase
- 3.5.1.58 N-Benzoyloxycarbonylglycine hydrolase
- 3.5.1.59 N-Carbamoylsarcosine amidase
- 3.5.1.72 D-Benzoylarginine-4-nitroanilide amidase
- 3.5.1.73 Carnitine amidase
- 15 3.5.1.75 Urethanase

Also preferred are enzymes of class 3.5.2 which act on cyclic amides, such as:

- 3.5.2.1 Barbiturase
- 3.5.2.2 Dihydropyrimidase
- 3.5.2.3. Dihydroorotase
- 20 3.5.2.4 Carboxymethylhydantoinase
- 3.5.2.5 Allantoinase
- 3.5.2.6 β -Lactamase
- 3.5.2.10 Creatininase

Also particularly preferred are class 3.5.3 enzymes which act on linear amidines, such

- 25 as:
- 3.5.3.1 Arginase
- 3.5.3.3 Creatinase
- 3.5.3.4 Allantoinase

- 3.5.3.6 Arginine deiminase
- 3.5.3.9 Allantoate deiminase
- 3.5.3.10 D-Arginase
- 3.5.3.14 Amidinoaspartase
- 5 3.5.3.15 Protein-arginine deiminase

Also particularly preferred are enzymes of class 3.5.4 which act on cyclic amidines, such as:

- 3.5.4.8 Aminoimidazolase
- 3.5.4.21 Creatinine deaminase

- 10 Preferred are also the enzymes of class 3.5.99 which act on other compounds, such as:
 - 3.5.99.1 Riboflavinase
 - 3.5.99.2 Thaminase

- 15 Particularly preferred enzymes are especially those of class 3.5.5. 1, nitrilase (3.5.5.2 - 3.5.5.6, other nitrilases).

Also particularly preferred are enzymes of class 3.5.1, here particularly those of class 3.5.1.4, amidases.

System Component 2 of the Enzyme Component System (ECS) of the Invention

- 20 Fatty acids which in the process according to the invention can be used as sources of peracids are, for example:

1) Saturated fatty acids

- 25 Butanoic acid (butyric acid)
- Pentanoic acid (valeric acid)
- Hexanoic acid (caproic acid)
- Heptanoic acid (enanthic acid)

Octanoic acid	(caprylic acid)
Nonanoic acid	(pelargonic acid)
Decanoic acid	(capric acid)
Undecanoic acid	
5 Dodecanoic acid	(lauric acid)
Tridecanoic acid	
Tetradecanoic acid	(myristic acid)
Pentadecanoic acid	
Hexadecanoic acid	(palmitic acid)
10 Heptadecanoic acid	
Octadecanoic acid	(stearic acid)
Nonadecanoic acid	
Eicosanoic acid	(arachic acid)
Heneicosanoic acid	
15 Docosanoic acid	(behenic acid)
Tricosanoic acid	
Tetracosanoic acid	(lignoceric acid)
Pentacosanoic acid	
Hexacosanoic acid	(cerotic acid)
20 Octacosanoic acid	
Triacontanoic acid	(melissic acid)

2) Unsaturated fatty acids

25 10-Undecenoic acid	
9-cis-Dodecenoic acid	(lauroleic acid)
9-cis-Tetradecenoic acid	(myristoleic acid)
9-cis-Hexadecenoic acid	(paimitoleic acid)
6-cis-Octadecenoic acid	(petroselic acid)
30 6-trans-Octadecenoic acid	(petroselaidic acid)
9-cis-Octadecenoic acid	(oleic acid)
9-trans-Octadecenoic acid	(elaidic acid)

9-cis, 12 cis-Octadecadienoic acid	(linoleic acid)
9-trans, 1 2-trans-Octadecadienoic acid	(linolaidic acid)
9-cis, 12-cis,15-cis-Octadecatrienoic acid	(linolenic acid)
9-trans, 11 -trans, 1 3-trans- Octadecatrienoic acid	(α -eleostearic acid)
5 9-cis, 11 -trans, 1 3-trans-Octadecatrienoic acid	(β -eleostearic acid)
9-cis-Icosenic acid	(gadoleic acid)
Icosa-5,8,11,14-tetraenoic acid	(arachidic acid)
13-cis-Docosenoic acid	(erucic acid)
13-trans-Docosenoic acid	(brassidic acid)
10 4,8,12,15,19-Docosapentaenoic acid	(clupanodonic acid)

3) Polyunsaturated fatty acids

9,12-Octadecadienoic acid	(linoleic acid)
15 9,12,1 5-Octadecatrienoic acid	(linolenic acid)
5,9,1 2-Octadecatrienoic acid	
9,11,13-Octadecatrienoic acid	(eleostearic acid)
9,11,13,15-Octadecatetraenoic acid	(parinarcic acid)
5,11,14-Icosatrienoic acid	
20 5,8,11,1 4-Icosatetraenoic acid	(arachidic acid)
4,8,12,15,1 8-Icosapentaenoic acid	
4,8,12,15,19-Docosapentaenoic acid	(clupanodonic acid)
4,8,12,15,18,21 -Tetracosahexaenoic acid	(nisinic acid)

25 Particularly preferred are tetradecanoic acid (myristic acid) and dodecanoic acid (lauric acid).

System Component 3 (Oxidants: Peroxides or Per Compounds) of the Enzyme Component System (ECS) of the Invention

Preferred oxidants in the enzyme component system of the invention are hydrogen peroxide (H_2O_2), organic peroxides and per-compounds such as perborates, persulfates, percarbonates, perphosphates, percarbamides, perchlorates etc.

Preferred organic peroxides are, for example:

5

3-chloroperoxybenzoic acid, monoperoxyphthalic acid Mg salt, di-tert.butyl peroxide, cumene hydroperoxide, lauroyl peroxide, chloroperoxybenzoic acid, dicumyl peroxide, methyl ethyl ketone peroxide, benzoyl peroxide, diperoxidododecandionic acid Na salt etc..

10 Besides lipase-catalyzed peracid formation, combinations of bleach activators, such as TAED (tetraacetylenediamine), TAGU (tetraacetylglycoluril) and iso-NOBS (sodium p-isobutyl-oxybenzenesulfonate) and the like, which are also used in detergents, together with per-compounds such as perborates, percarbonates etc. can serve as additional sources of peracid generation.

15 The abovesaid per-compounds, as well as, for example, glucose + GOD, can be used as systems generating H_2O_2 for the corresponding lipase action. Substances such as nitrilamines or dicyandiamines or metal ions, e.g. Mo^{6+} , V^{6+} and W^{6+} can be used together with a peroxide, for example H_2O_2 .

20 **System Component 4 (Ketones) of the Enzyme Component System (ECS) of the Invention**

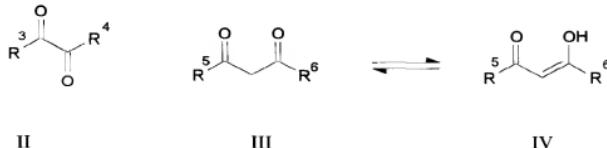
Particularly preferred are carbonyl compounds of general formula I:

25



30 The R^1 and R^2 groups can be equal or different and denote aliphatic or aromatic groups. Moreover, the R^1 and R^2 groups can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen and sulfur.

Particularly preferred are 1,2-diketones (formula II), 1,3-diketones (formula III), polyketones (polyketides) and the tautomeric enols (formula IV):



10 wherein the R^3 to R^6 groups, once again, can be equal or different and denote aliphatic or aromatic groups. Moreover, groups R^3 and R^4 and groups R^5 and R^6 , together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur. The possibility of tautomerization or formation of a resonance hybrid is particularly important in this case.

15 Besides general carbonyl compounds, particularly preferred are ketones, such as, in general hydroxyketones, α,β -unsaturated ketones, oxycarboxylic acids, quinones and halogenated ketones.

Particularly preferred among these are the following:

20 Acetone, methyl ethyl ketone, diethyl ketone, methyl n-butyl ketone, methyl isobutyl ketone, cyclohexanone, cyclopentanone, 2-methylcyclohexanone, 3-methylcyclohexanone, 4-methylcyclohexanone, dihydroxyacetone, diacetyl monohydrazone, diacetyl dihydrazone, acetophenone, p-hydroxyacetophenone,

25 1 -phenyl-3-butanone, 3-pentanone, 4-heptanone, 2-nonanone, cycloheptanone, cyclooctanone, cyclodecanone, cyclododecanone, dimethyl ketone, ethyl propyl ketone, methyl amyl ketone, acetylacetone, pinacoline, methyl isopropyl ketone, methyl isoamyl ketone, ethyl amyl ketone diisopropyl ketone, diisobutyl ketone, methyl vinyl ketone, methyl isopropenyl ketone, mesityl oxide, isophorone, hydroxyacetone,

methoxyacetone, 2,3-pentanedione, 2,3-hexanedione, phenylacetone, propiophenone,
benzophenone, benzoin, benzil, 4,4'-dimethoxybenzil, 4'-methoxyacetophenone,
3'- methoxyacetophenone, O-ethylbenzoin, (2-methoxyphenyl)acetone, (4-
methoxyphenyl)acetone, methoxy-2-propanone, glyoxylic acid, benzyl glyoxylate,
5 benzylacetone, methyl benzyl ketone, methylcyclohexyl ketone, 2-decanone,
dicyclohexyl ketone, 3,3-dimethyl-2-butanone, methyl isobutyl ketone, methyl
isopropyl ketone, 2-methyl-3-heptanone, 5-methyl-3-heptanone, 6-methyl-5-hepten-2-
one, 5-methyl-2-hexanone, 3-nonanone, 5-nonanone, 2-octanone, 3-octanone, 2-
undecanone, 1,3- dichloroacetone, 1-hydroxy-2-butanone, 3-hydroxy-2-butanone, 4-
10 hydroxy-4-methyl-2-pentanone, 2-(1S)- adamantanone, anthrone, bicyclo(3.2.0)hept-2-
en-6-one, cis-bieyclo(3.3.0)octan-3,7-dione, (1S)- (-)-camphor, p-chloranil,
cyclobutanone, 1,3-cyclohexanedione, 1,4-cyclohexanedione monoethylene ketal,
dibenzosuberone, ethyl 4-oxocyclohexanecarboxylate, 9-fluorenone, 1,3-indandione,
methyl- cyclohexanone, phenylcyclohexanone, 4-propylcyclohexanone, 1,2,3,4-
15 tetrahydro-1-naphthalenone, 1,2,3,4-tetrahydro-2-naphthalenone, 3,3,5-trimethylcyclo-
hexanone, 3-acetoxy-2-cyclohexen-1-one, benzylideneacetone, (R)-(-)-carvone,
(S)-(-)carvone, curcumin, 2-cyclohexen-1-one, 2,3-diphenyl-2- cyclopropen-1-one, 2-
hydroxy-3-methyl-2-cyclopentene-1-one, isophorone, α -ionone, β -ionone, 3-methoxy-2-
cyclohexen-1-one, 3-methyl-2-cyclopenten-1-one, 3-methyl-3-penten-2-one, (R)-(+)-
20 pulegone, tetraphenyl-2,4-cyclopentadien-1-one, 2,6,6-trimethyl-2-cyclohexen-1,4-
dione, 2-acetylbenzoic acid, 1-acetyl naphthalene, 2-acetyl naphthalene,
3'-aminoacetophenone, 4'-aminoacetophenone, 4'-cyclohexylacetophenone, 3',4'-
diacetoxyacetophenone, diacetylbenzene, 2',4'-dihydroxyacetophenone, 2',5'-
dihydroxyacetophenone, 2',6'-dihydroxyacetophenone, 3,4-dimethoxyacetophenone,
25 2'-hydroxyacetophenone, 4'-hydroxyacetophenone, 3'-methoxyacetophenone, 4'-
methoxyacetophenone, 2'-methylacetophenone, 4'-methylacetophenone,
2'-nitroacetophenone, 3'-nitroacetophenone, 4'-phenylacetophenone, 3',4',5'-trimethoxy-
acetophenone, 4'-aminopropiophenone, benzoylacetone, benzoylpropionic acid,
benzylideneacetophenone, cyclohexyl phenyl ketone, desoxybenzoin, 4',4'-
30 dimethoxybenzil, 1,3-diphenyl-1,3-propanedione, ethylbenzoyl acetate, ethyl
phenylglyoxylate, 4'- hydroxypropiophenone, 1,3-indandione, 1-indanone, isopropyl
phenyl ketone, 6-methoxy-1,2,3,4- tetrahydronaphthalen-1-one, methylphenyl

glyoxylate, phenylglyoxylonitrile, 1-phenyl-1,2-propanedione 2-oxime, valerophenone,
2-acetyl- γ -butyrolactone, 2-acetylpyrrole, 1-benzylpiperidin-4-one, dehydroacetic acid,
3,4-dihydro-4,4-dimethyl-2H-pyran-2-one, 1,4-dihydro-4-pyridinone, N-eth-
oxycarbonyl-4-piperidinone, 2-methyl furyl ketone, 5-hydroxy-2-hydroxymethyl-4H-
5 pyran-4-one, 3-hydroxy-2-methyl-4-pyranone, 3-indolyl methyl ketone, isatin, 1-
methyl-4-piperidinone, methyl 2-pyridyl ketone, methyl 3-pyridyl ketone, methyl 4-
pyridyl ketone, methyl 2-thienyl ketone, phenyl 2-pyridyl ketone, phenyl 4-pyridyl
ketone, tetrahydrofuran-2,4-dione, tetrahydro-4H-pyran-4-one, 2,2,6,6-tetramethyl-4-
piperidone, xanthone, acenaphthene quinone, pyruvic acid, (1 R)-(-)-camphor quinone,
10 (IS)-(+)-camphor quinone, 3,5-ditert.butyl-o-benzoquinone, 1,2-dihydroxy-3,4-
cyclobutendione, ethyl (2-amino-4-thiazolyl)glyoxylate, ethyl pyruvate, 2,3-
hexanedione, 3,4- hexanedione, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric
acid, 4-methyl-2-oxovaleric acid, 2- oxobutyric acid, 2,3-pentandione, 9,10-
phenanthrene quinone, acetoacetanilide,2-acetyl- γ -butyrolactone, 2-acetylcyclo-
15 pentanone, allyl acetoacetate, benzoylacetone, tert.butyl acetoacetate, 1,3-
cyclopentanedione, diethyl 3-oxoglutarate, dimethyl acetylsuccinate, dimethyl 3-
oxoglutarate, 1,3-diphenyl-1,3-propanedione, ethyl acetoacetate, ethyl benzoylacetate,
ethyl butyrylacetate, ethyl 2-oxocyclohexanecarboxylate, ethyl 2-phenylacetacetate,
methyl acetoacetate, 2-methyl-1,3- cyclohexanedione, 2-methyl-1,3-cyclopentanedione,
20 methyl isobutyrylacetate, methyl 3-oxopentanoate, methyl pivaloylacetate, 3-
oxoglutaric acid, tetrahydrofuran-2,4-dione, 2,2,6,6-tetramethyl-3,5-
heptanedione, 3-benzoylpropionic acid, 1,4-cyclohexanedione, dimethyl
acetylsuccinate, ethyl levulinic, 2-aminoanthraquinone, anthraquinone, p-
benzoquinone, 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone,
25 2-ethylanthraquinone, methyl-p-benzoquinone, 1,4-naphthoquinone, tetramethyl-p-
benzoquinone, 2,2-dimethyl-1,3-dioxan-4,6-dione, 2-benzoylbenzoic acid, 3-benzoyl-
propionic acid, 5,6-dimethoxyphthalaldehydic acid, levulinic acid, methyl trans-4-oxo-
2-pentenoate, phthalaldehydic acid, terephthalaidehydic acid, dibutyl maleate, dibutyl
succinate, dibutyl phthalate, dicyclohexyl phthalate, diethyl acetamidomalonate, diethyl
30 adipate, diethyl benzylmalonate, diethyl butylmalonate, diethyl ethoxymethylene-
malonate, diethyl ethylmalonate, diethyl fumarate, diethyl glutarate, diethyl
isopropylidenemalonate, diethyl maleate, diethyl malonate, diethyl methylmalonate,

diethyl oxalate, diethyl 3-oxoglutarate, diethyl phenylmatonate, diethyl phthalate,
diethyl pimelate, diethyl sebacate, diethyl suberate, diethyl succinate, diisobutyl
phthalate, dimethyl acetylene- dicarboxylate, dimethyl acetylsuccinate, dimethyl
adipate, dimethyl 2-aminoterephthalate, dimethyl fumarate, dimethyl glutaconate,
5 dimethyl glutarate, dimethyl isophthalate, dimethyl malonate, dimethylmethoxy-
malonate, dimethyl methylenesuccinate, dimethyl oxalate, dimethyl 3-oxoglutarate,
dimethyl phthalate, dimethyl succinate, dimethyl terephthalate, ethylene glycol
diacetate, ethylene glycol dimethacrylate, monoethyl fumarate, monomethyl malonate,
monoethyl adipate, monomethyl phthalate, monomethyl pimelate, monomethyl
10 terephthalate, 1,2-propylene glycol diacetate, triethyl methanetricarboxylate, trimethyl
1,2,3-propanetricarboxylate, 3-acetoxy-2-cyclohexen-1-one, allyl acetoacetate, allyl
cyanoacetate, benzyl acetoacetate, tert.butyl acetoacetate, butyl cyanoacetate,
chlorogenic acid hemihydrate, coumarin-3-carboxylic acid, diethyl ethoxy-
carbonylmethanephosphonate, dodecyl gallate, dodecyl 3,4,5-trihydroxybenzoate, (2,3-
15 epoxypropyl) methacrylate, (2-ethoxyethyl) acetate, ethyl acetamidocynoacetate, ethyl
2-aminobenzoate, ethyl 3-aminopyrazol-4-carboxylate, ethyl benzoxylacetate, ethyl
butyrylacetate, ethyl cyanoacetate, ethyl 2-cyano-3-ethoxyacrylate, ethyl cyanoformate,
ethyl 2-cyanopropionate, ethyl 3,3-diethoxypropionate, ethyl 1,3-dithian-2-carboxylate,
ethyl 2-ethoxyacetate, ethyl 2-furancarboxylate, ethyl levulinate, ethyl mandelate, ethyl
20 gallate, ethyl 2-methyllactate, ethyl 4- nitrocinnamate, ethyl oxamate, ethyl 2-
oxocyclohexanecarboxylate, ethyl 4-oxocyclohexane- carboxylate, ethyl 5-
oxohexanoate, ethyl 2-phenylacetocetate, ethyl 4-piperidinecarboxylate,
ethyl 2-pyridinecarboxylate, ethyl 3-pyridinecarboxylate, ethyl 4-
25 pyridinecarboxylate, ethyl thioglycolate, ethyl 3,4,5-trihydroxybenzoate, 2-
hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, 3-indole acetate, 2-
methoxyethyl acetate, 1-methoxy-2-propyl acetate, methyl 2- aminobenzoate, methyl 3-
aminocrotonate, methyl cyanoacetate, methyl 4-cyanobenzoate, methyl 4-
30 formylbenzoate, methyl 2-furancarboxylate, methyl isobutyrylacetate, methyl
methoxyacetate, methyl 2-methoxybenzoate, methyl 3-oxopentanoate, methyl
phenylglyoxylate, methyl phenyl- sulfinylacetate, methyl pivatoylacetate, methyl 3-
pyridinecarboxylate, 5-nitrofurylidene diacetate, propyl gallate, propyl 3,4,5-
trihydroxybenzoate, methyl 3-methylthiopropionate, acetamide, acetani-

lide, benzamide, benzanilide, N,N-diethylacetamide, N,N-dimethylformamide, N,N-diethyl-3-methyl- benzamide, diethyltoluamide, N,N-dimethylacetamide, N,N-diphenylacetamide, N-methylformamide, N-methylformanilide, N-acetylthiourea, adipic acid diamide, 2-aminobenzamide, 4-aminobenzamide, succinic acid diamide, malonic acid diamide, N,N'-methylene diacrylamide, oxalic acid diamide, pyrazine-2-carboxamide, pyridine-4-carboxamide, N,N,N',N'-tetramethylsuccinic acid diamide, N,N,N',N'-tetramethylglutaric acid diamide, acetoacetanilide, benzohydroxamic acid, cyanoacetamide, 2-ethoxybenzamide, diethyl acetamidomalonate, ethyl acetamidocyanacetate, ethyl oxamate, hippuric acid Na salt, N-(hydroxy-10-methyl)acrylamide, L-(-)-lactamide, 2'-nitroacetanilide, 3'- nitroacetanilide, 4'-nitroacetanilide, paracetamol, piperine, salicylanilide, 2-acetyl- γ -butyrolactone, γ -butyrolactone, ϵ -caprolactone, dihydrocoumarin, 4-hydroxycoumarin, 2-(5H)-furanone, 2,5-dihydro-5-methoxy-2-furanone, phthalide, tetrahydrofuran-2,4-dione, 2,2,6-trimethyl-1,3-dioxin-4-one, γ - valerolactone, 4-amino-1,3-dimethyluracil, barbituric acid, O-benzyloxycarbonyl-N-hydroxysuccinimide, succinimide, 3,6-dimethyl-piperazin-2,5-dione, 5,5-diphenylhydantoin, ethyl 1,3-dioxoisindoline-2-carboxylate, 9-fluorenylmethylsuccinimidyl carbonate, hydantoin, maleimide, 3-methyl-L- phenyl-2-pyrazolin-5-one, 1-methyl-2-pyrrolidone, methyluracil, 6-methyluracil, oxindole, phenytoin, 1-(2H)-phthalazinone, phthalimide, 2,5-piperazinedione, 2-piperidinone, 2-pyrrolidone, rhodanine, saccharin, 1,2,3,6-tetrahydrophthalimide, 1,2,3,4-tetrahydro-6,7-dimethoxyquinazolin-2,4-dione, 1,5,5-trimethyl-hydantoin, 1-vinyl-2-pyrrolidone, ditert.butyl dicarbonate, diethyl carbonate, dimethyl carbonate, dimethyl dicarbonate, diphenyl carbonate, 4,5-diphenyl-1,3-dioxol-2-one, 4,6- diphenylthieno-(3,4-d)-1,3-dioxo-2-one 5,5-dioxide, ethylene carbonate, magnesium methoxide methyl carbonate, monomethyl carbonate Na salt, propenyl carbonate, N-allylurea, azodicarbonamide, N-benzylurea, biuret, 1,1'-carbonyldiimidazol, N,N-dimethylurea, N-ethylurea, N-formylurea, urea, N-methylurea, N-phenylurea, 4-phenylsemicarbazide, tetramethylurea, semicarbazide hydrochloride, diethyl azodicarboxylate, methyl carbamate, 1-(4-methoxyphenyl)-2-(2- methoxyphenoxy)ethanone and 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanol.

Also preferred are anhydrides, such as the following:

Benzoic anhydride, benzene-1,2,4,5-tetracarboxylic acid-1,2,4,5-dianhydride, 3,3',4,4'-benzophenonetetracarboxylic anhydride, succinic anhydride, butyric anhydride, crotonic anhydride, cis-1,2-cyclo- hexanedicarboxylic anhydride, ditert.butyl dicarbonate, dimethyl dicarbonate, dodecenylsuccinic anhydride, Epicon B 4400, acetic anhydride,

5 glutaric anhydride, hexanoic anhydride, isatoic anhydride, isobutyric anhydride, isovaleric anhydride, maleic anhydride, 1,8-naphthalenedicarboxylic anhydride, 3-nitrophthalic anhydride, 5-norbornene-2,3-dicarboxylic anhydride, phthalic anhydride, 2-phenylbutyric anhydride, pivalic anhydride, propionic anhydride, cis-1,2,3,6-tetrahydrophthalic anhydride and valeric anhydride.

10

Particularly preferred are benzophenones such as the following:

Benzophenone, 4-aminobenzophenone, 2-amino-5-chlorobenzophenone, benzophenone-2-carboxylic acid, (S)-(-)-2-(N-benzopropyl)aminobenzophenone, 4,4'-bis(dimethylamino)benzophenone, 4,4'- bis(diethylamino)benzophenone, 3,4-dimethoxybenzophenone, 4,4'-dihydroxybenzophenone, 2,4- dihydroxybenzophenone, 4-hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 4,4'-dimethoxybenzophenone, 2,2',4,4'-tetrahydroxybenzophenone and 2-chlorobenzophenone.

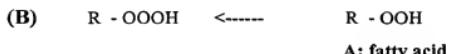
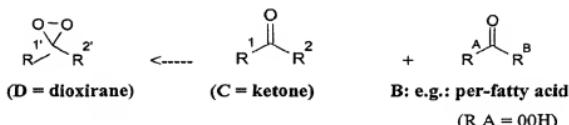
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Figure 1 shows schematically a possible reaction cycle involving all components.

Figure 1:



lipase/amidase

component 1) = enzyme/ hydrolase: e.g.: lipase/amidase

component 2) = fatty acid (A)

component 3) = oxidation agent, e.g.: (Oxi)

component 4) = ketone (C)

B) = e.g.: per-fatty acid

D) = dioxirane

A more detailed description of the enzyme component system (ECS) of the invention in terms of various applications follows:

1) Use in Wood Pulp Bleaching

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One of the components of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 5 mg per gram of wood pulp, preferably from 0.05 to 2 mg of enzyme per gram of wood pulp (which corresponds to about 250 to 10,000 IU per gram of wood pulp) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

Preferably, the delignification (bleaching) with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slight positive O₂ pressure and at pH from 2 to 11, preferably at pH 3-9, at a

15 temperature of 20 to 95 °C, preferably 40-95 °C and a pulp consistency of 0.5 to 40%.

An unusual and surprising finding concerning the use of enzymes for wood pulp bleaching is that when the enzyme component system of the invention is used, the consistency of the material can be increased and the kappa value thus markedly reduced.

For economic reasons, the process according to the invention is carried out at a pulp consistency from 4 to 35% and particularly from 4 to 15%.

Another component is the oxidant, preferably H₂O₂, which is used at a concentration from 0.05 to 20 mg/g of wood pulp (100% basis) and preferably from 0.05 to 10 mg/g of wood pulp.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆, and particularly C₆ to C₁₆ fatty acids and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 20 mg/g of wood pulp and preferably at a concentration from 0.05 to 10 mg/g of wood pulp.

Another component are the ketones, preferably, for example, benzophenone at a concentration from 0.05 to 20 mg/g of wood pulp and preferably at a concentration from 0.05 to 10 mg/g of wood pulp.

When the enzyme component system of the invention is used, for example, in a process for treating lignin, the chosen components are mixed with an aqueous suspension of the

lignin-containing pulp simultaneously or in any order. The reaction is preferably started by adding the oxidant or the enzyme.

Besides the abovesaid main components of the enzyme component system (ECS) of the invention, namely the enzymes (lipases/amidases), the oxidant, the fatty acids and the 5 ketones, the bleaching system can also contain phenolic and/or nonphenolic compounds with one or more benzene rings which are capable of improving "oxidation transfer" (redox cascade) and/or of scavenging the radicals which possibly could cause polymerization of the lignin.

Besides the abovesaid preferred oxidant, H_2O_2 , particularly preferred are air, oxygen 10 (possibly in addition to H_2O_2), organic peroxides, percompounds such as sodium perborate and/or sodium percarbonate, persulfates etc. (optionally together with activators such as TAED, nitrilamines, dicyandiamines etc.). Oxygen can also be generated in situ by $H_2O_2 +$ catalase or the like, or H_2O_2 , can be generated in situ from GOD + glucose or similar systems.

15 The efficacy of the enzyme component system (ECS) as the oxidation system of the invention in the modification, degradation or bleaching of lignin, lignin-containing materials or similar substances is often even enhanced when Mg^{2+} ions are present besides the said constituents. The Mg^{2+} ions can be derived, for example, from a salt such as $MgSO_4$. The Mg^{2+} concentration is in the range from 0.1 to 2 mg/g, and 20 preferably from 0.2 to 0.6 mg/g, of lignin-containing material.

In some cases, the efficacy of the enzyme component system (ECS) of the invention can be increased even further by adding to the system besides Mg^{2+} ions also complexing 25 agents, for example ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydroxyethylenediaminetriacetic acid (HEDTA), diethylenetriaminepentamethylenephosphonic acid (DTMPA), nitrilotri-acetic acid (NTA), polyphosphoric acid (PPA) etc. The concentration of said complexing agents is in the range from 0.2 to 5 mg/g, and preferably from 1 to 3 mg/g, of the lignin-containing material.

Surprisingly, we have also found that for many wood pulps an acid wash (pH 2 to 6 and 30 preferably 2 to 5) or a Q step (pH 2 to 6 and preferably 2 to 5) preceding the ECS step causes a marked decrease in kappa value compared to processing without this special pretreatment. In the Q step, chelators usually employed for this purpose (for example,

EDTA or DTPA) are preferably used at a concentration from 0.1 to 1 % and particularly from 0.1 to 0.5 %.

Reducing agents can be added at the same time, which with the oxidants present give rise to a certain redox potential. Suitable reducing agents are sodium bisulfite, sodium

5 dithionite, ascorbic acid, thio compounds, mercapto compounds or glutathione etc..

It is also possible to add to the system radical generators or radical scavengers (scavenging, for example, OH- or OOH- radicals). These can improve the interaction between the redox and the radical mediators.

Additional metal salt can also be added to the reaction solution. These are important in 10 that they interact with chelators as radical generators or redox centers. In the reaction solution, the salts form cations. Such ions are, among others, Fe^{2+} , Fe^{3+} , Mn^{2+} , Mn^{3+} , Mn^{4+} , Cu^{1+} , Cu^{2+} , Ca^{2+} , Ti^{3+} , Ce^{4+} and Al^{3+} .

The chelates present in the solution can also serve as mimicking substances for certain 15 oxido- reductases, such as the laccases (copper complexes) or for the lignin or manganese peroxidases (heme complexes). By mimicking substances are meant substances simulating the prosthetic groups of (in the present case) oxidoreductases and, for example, capable of catalyzing oxidation reactions.

Moreover, NaOCl can be added to the reaction mixture. This compound can form singlet oxygen by interacting with hydrogen peroxide.

20 Finally, it is also possible to use detergents. These include nonionic, anionic, cationic and amphoteric surfactants. Detergents improve the penetration of the enzymes and other components into the fibers.

It may also be necessary to add polysaccharides and/or proteins to the reaction mixture. 25 Suitable polysaccharides are, in particular, glucans, mannans, dextrans, levans, pectins, alginates or vegetable gums, and suitable proteins are gelatins and albumins. These substances serve mainly as protective colloids for the enzymes.

Other proteins that can be added are proteases such as pepsin, bromelain, papain etc.

These substances can, among other things, bring about the degradation of extensin (hydroxyproline-rich protein) present in wood, thus improving access to the lignin.

30 Other suitable protective colloids are amino acids, monosaccharides, oligosaccharides, polyethylene glycol [PEG] types of a wide range of molecular weights, polyethylene oxides, polyethyleneimines and polydimethylsiloxanes.

It is also possible to add to the enzyme component system of the invention substances capable of increasing the hydrophobicity of the reaction mixture, thus bringing about the swelling of the lignin and the fibers and which makes them more susceptible to attack. Such substances are, for example, glycols, such as propylene glycol and ethylene

5 glycol, glycol ethers such as ethylene glycol dimethyl ether etc., and solvents, for example, alcohols such as methanol, ethanol, butanol, amyl alcohol, cyclohexanol, benzyl alcohol and chlorohydrin, phenols such as phenol, methylphenols and methoxyphenols, aldehydes such as formaldehyde and chloral, mercaptans such as butyl mercaptan, benzyl mercaptan and thioglycolic acid, organic acids such as formic, acetic 10 and chloroacetic acid, amines such as ammonia and hydrazine, hydrotropic solvents, for example concentrated solutions of sodium benzoate, other substances such as benzenes, pyridines, dioxane, ethyl acetate, and other basic solvents such as OH/ H₂O or OH⁻ /alcohol etc..

The process according to the invention can be used not only for the delignification

15 (bleaching) of sulfate, sulfite, organosolv or other wood pulps or lignins, but also for the preparation of wood pulp in general, whether from wood or annual plants, when it is desired to carry out the defibrillation by the usual cooking (digestion) process (possibly combined with mechanical processing or pressure), namely by very gentle digestion, up to kappa numbers in the range from about 50 - 120 kappa.

20 In the bleaching as in the preparation of wood pulps, the treatment with the enzyme component system (ECS) of the invention can be applied once or several times, either before and/or after the washing and extraction of the treated material with NaOH etc., or without these intermediate steps, but also before and/or after pretreatment or post-treatment steps, such as acid washing, Q-steps, alkaline leaching or bleaching steps such 25 as peroxide bleaching, O₂-enhanced peroxide steps, pressurized peroxide steps, O₂-delignification, Cl₂ -bleaching, ClO₂ -bleaching, Cl₂/ClO₂-bleaching, peracid bleaching, peracid-enhanced O₂/peroxide bleaching, ozone bleaching, dioxirane bleaching, reductive bleaching steps, other treatments such as swelling steps, sulfonations, NO/NO₂ treatments, nitrosylsulfuric acid treatment, enzyme treatments, 30 for example treatments with hydrolases, such as cellulases and/or hemicellulases (for example, xylanase, mannase etc) and/or amylases and/or pectinases and/or proteinases

and/or lipases and/or amidases and/or oxidoreductases such as, for example, laccases and/or peroxidases etc., or several combined treatments.

This results in substantially further reduced kappa values and substantially increased brightness. Before the ECS treatment, it is also possible to insert an O₂ step or, as 5 already mentioned, carry out an acid wash or a Q-step (chelation step).

The invention will be further illustrated by way of the following examples:

EXAMPLE 1

Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30%

10 (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

15 B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 20 hours under atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% consistency and using 8% NaOH per gram of wood pulp. The material was again washed after which the kappa number was determined. For results see Table 1.

25 **EXAMPLE 1a**

Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or 5 sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

10 Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

15 The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

EXAMPLE Ib (+ H₂O₂, with Nitrilamine as Activator)

Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% 20 (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone, 2.5 mg of H₂O₂ (30%) and 0.5 mg of nitrilamine with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

25 B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

5 The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2 % pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

EXAMPLE 2

10 **Enzymatic Bleaching of O₂-delignified Hardwood (Sulfate Pulp)**

5 g, absolutely dry basis, of wood pulp (O₂-delignified hardwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with 15 sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

20 Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2 % pulp consistency and using 8% NaOH per gram of wood pulp. The material was 25 again washed after which the kappa number was determined. For results see Table 1.

EXAMPLE 2a

5 g, absolutely dry basis, of wood pulp (O₂-delignified hardwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

5 A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of acetone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU).

10 Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

15 The material was washed over a nylon screen (30 µm) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was again washed, after which the kappa number was determined. For results see Table 1.

EXAMPLE 3**Enzymatic Bleaching of O₂-delignified Softwood (Sulfate Pulp)**

20 5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows.

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone and 2.5 mg of H₂O₂ (30%) with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the wood pulp 25 and the enzyme, the pH was 7.5.

B) To 5 mL of tap water was added 200 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691) (1 IU = conversion of 1 μ mole of acetamide and hydroxylamine to acetohydroxamic acid and NH₃ per minute at pH 7.2 and 37 °C).

Solutions A and B were combined and diluted to 33 mL. After addition of the wood

5 pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and was allowed to incubate 1-4 hours at atmospheric pressure.

The material was washed over a nylon screen (30 μ m) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The material was 10 again washed, after which the kappa number was determined. For results see Table 1.

TABLE 1

	Wood pulp	% Delignification (before extraction)	% Delignification (after extraction)
15	a) Softwood (untreated)	----	5.8%
20	b) Softwood (lipase-treated)	19% <u>17.5%</u>	32.0% <u>31%*</u> <u>35%**</u>
25	c) Hardwood (untreated)	-----	6.5%
	d) Hardwood (lipase-treated)	21% <u>18%*</u>	33% <u>28%*</u>
30	e) Softwood (amidase-treated)	15.5%	23%
	f) Comparative example: laccase + HOBT 5 kg/ton of wood pulp, other conditions as in WO 96/18770 (pulp a/b)	17.5%	22%

*Underlined values were obtained with acetone as the ketone.

35 **Value obtained with added nitrilamine.

II) Use in Enzymatic Wastewater Treatment, for Example of Grinder Wastewater in the Paper Industry

Because in this application the polymerization of lignin or lignin constituents contained in the wastewater is desired rather than lignin degradation, the enzyme component

5 system (ECS) of the invention is used with a small amount of added polymerization catalyst.

One component of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from *Aspergillus* spec., at a concentration from 0.05 to 50 mg per liter of wastewater and preferably from 0.05 to 10 mg of enzyme per liter of

10 wastewater (corresponding to about 250 to 50,000 IU per liter of wastewater) (1 IU hydrolyzes 1 μ equivalent of triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

The treatment of the grinder wastewater with the enzyme component system of the invention is preferably carried out in the presence of oxygen or air at atmospheric pressure or slight positive oxygen pressure and at a pH from 2 to 11 and preferably from 15 3 to 6, at a temperature from 20 to 95 °C and preferably from 40 to 95 °C.

Another component is the oxidant, preferably H_2O_2 , which is used at a concentration from 0.05 to 200 mg per liter of wastewater (100% basis) and preferably from 0.05 to 50 mg per liter of wastewater.

Another component consists of one or more fatty acids, preferably C_6 to C_{26} and 20 particularly C_6 to C_{16} , fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 200 mg per liter, and preferably at a concentration from 0.05 to 10 mg per liter, of wastewater.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 200 mg per liter of wastewater and preferably at a 25 concentration from 0.05 to 50 mg per liter of wastewater.

Moreover, to increase the efficiency of the process and to use less precipitant (mostly sodium aluminate/aluminum sulfate) which represents the main cost factor, a polymerization catalyst is used, mostly a phenolic substance or a polycyclic compound

with several oxidizable hydroxyl groups, in our case preferably, for example, purpurogallin.

These substances are used at a concentration from 0.005 to 200 mg per liter of wastewater and preferably at a concentration from 0.005 to 50 mg per liter of wastewater.

The invention will be further illustrated by way of the following examples:

EXAMPLE 4

190 mL of grinder wastewater was adjusted to pH 6, its temperature was adjusted to 45 °C in an appropriate jacketed reaction vessel and to it were added the following

10 solutions:

- 1) Enzyme solution: lipase (*Aspergillus spec.*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water
- 15 4) Polymerization catalyst: 0.1 mg of purpurogallin in 0.1 mL of water.

The reaction was initiated by addition of solution 5) (oxidant: H_2O_2), namely of a solution of 3.3 mg of H_2O_2 (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and 20 precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case and based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system.

25 The results are collected in Table 2.

EXAMPLE 5 (without polymerization catalyst)

190 mL of grinder wastewater was adjusted to pH 6, its temperature was adjusted to 45 °C in an appropriate jacketed reaction vessel and to it were added the following solutions:

- 5 1) Enzyme solution: lipase (*Aspergillus* spec.): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water

The reaction was initiated by addition of solution 4) (oxidant- H₂O₂), namely a solution of 3.3 mg of H₂O₂ (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL with preheated water. The reaction was allowed to proceed for 1 to 4 hours and

- 10 10) preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin
- 15 15) is a measure of COD reduction and of the efficiency of the system.

The results are collected in Table 2.

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35

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TABLE 2**Treatment****Residual Lignin After 2 Hours
(0 Value = 600 mg/liter)**

5

COD (mg/L)No treatment
(filtered)

800

No treatment
(filtered/precipitated*)

720

10

With treatment (lipase)
(filtered/precipitated),
(with polymerization cat.)*

100

15

Comparative treatment:
with 25,000 IU of laccase
per liter of wastewater
(filtered/precipitated*)
(with polym. catalyst)**

170

20

With treatment (lipase)
(filtered/precipitated*)
(without polym. catalyst)

220

25

With treatment
(filtered/precipitated*)
with polym. catalyst
(Humicola lipase)
(For the test with polymerization
and/or modification of lignin, see below)

160

* Only the precipitations at 0.5%/0.5% are shown.

30

** Polymerization catalyst

III) Use in the Preparation of Lignin Solutions or Gels, Corresponding Binders/Adhesives and of Wood-Based Composites

In this application, too, the polymerization of the lignin or lignin-containing materials is desired and not lignin degradation. Hence, the enzyme component system (ECS) of the invention is used with a small amount of added polymerization catalyst.

Because it was found that the polymerization of lignin, for example in groundwood pulp wastewater (grinder wastewater) is also a good system for evaluating general polymerization properties for the use of the enzyme component system (ECS) in the preparation of lignin solutions or gels, of the corresponding binders/adhesives and of wood-based composites, tests were carried out with the same experimental formulations as for the wastewater.

In this regard, it is known from the cited patents WO 94/01488, WO 93/25622, WO 93/23477 and DE 3 037 992 C2 that, for example, in the production of particle board the binder made by polymerization and dissolution of lignin is applied by spraying it onto the wood fiber material in an amount of about 40 to 100 g per kg of said material which is then subjected to pressing at a pressure of about 20-40 kg / cm² for about 2-4 min and at a temperature rising from about 35 to 190 °C within about 20 seconds. The pressures and temperatures used for pressing can, of course, also be substantially lower, and subsequent curing of the binder/wood fiber mixture by continuing enzyme-catalyzed reactions may be desired.

To evaluate the polymerization properties of ECS for this application, we used as the model system, as stated hereinabove, the above-described system for removing lignin from grinder wastewater.

As one component of the enzyme component system (ECS) of the invention was used an enzyme, preferably lipase from *Humicola lanuginosa*, at a concentration from 0.05 to 50 mg per liter of wastewater and preferably from 0.05 to 10 mg of enzyme per liter of wastewater (corresponding to about 250 to 50,000 IU per liter of wastewater) (1 IU hydrolyzes 1 μ equivalent of triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). The treatment of the grinder wastewater with the enzyme component system of the invention is preferably carried out in the presence of oxygen or air at atmospheric

pressure or slight positive oxygen pressure and at a pH from 2 to 11 and preferably from 3 to 6, at a temperature from 20 to 95 °C and, preferably from 40 to 95 °C.

Another component is the oxidant, preferably H₂O₂ which is used at a concentration from 0.05 to 200 mg per liter of wastewater (100% basis) and preferably from 0.05 to 50 mg/liter of wastewater.

Another component consists of one or more fatty acids, preferably a C₆ to C₂₆, particularly C₆ to C₁₆ fatty acid and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 200 mg/liter of wastewater and preferably at a concentration from 0.05 to 50 mg/liter of wastewater.

10 Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 200 mg/liter of wastewater and preferably at a concentration from 0.05 to 50 mg/liter of wastewater.

Moreover, as already stated in the foregoing, to increase the efficiency of the process a polymerization catalyst is used, mostly a phenolic substance or a polycyclic compound 15 with several oxidizable hydroxyl groups, in our case preferably, for example, purpurogallin.

These substances are used at a concentration from 0.005 to 200 mg per liter of wastewater and preferably at a concentration from 0.005 to 50 mg per liter of wastewater.

20

EXAMPLE 6

190 mL of grinder wastewater was adjusted to pH 8.5, preheated to a temperature of 45 °C in an appropriate jacketed reaction vessel and to it were added the following 25 solutions:

- 1) Enzyme solution: lipase (*Humicola lanuginosa*): 1 mg in 0.1 mL of water
- 2) Fatty acid solution: 1 mg of dodecanoic acid in 1 mL of water
- 3) Ketone solution: 1 mg of 2,2',4,4'-tetrahydroxybenzophenone in 1 mL of water
- 4) Polymerization catalyst: 0.1 mg of purpurogallin in 0.1 mL of water.

30

The reaction was initiated by addition of solution 5) (oxidant: H₂O₂), namely a solution of 3.3 mg of H₂O₂ (30%) in 0.1 mL of water, and the volume was adjusted to 200 mL.

with preheated water. The reaction was allowed to proceed for 1 to 4 hours and preferably for 2 hours. The wastewater was then either only filtered or filtered and precipitated with 0.2%/0.2% or 0.5%/0.5% aluminum sulfate solution/sodium aluminate solution, 10 wt % in each case, based on the initial value without treatment. The lignin which in untreated grinder wastewater is usually present in an amount from 600 to 900 mg per liter was determined quantitatively by photometry at 280 nm. The drop in lignin is a measure of COD reduction and of the efficiency of the system.

5 The results are collected in Table 2.

10

IV) Use as Enzymatic Deinking System

In this application, no lignin degradation is desired but, rather, a swelling effect on the lignin-containing fibers so as to bring about the detachment of adhering printing ink
15 particles, an effect similar to that of sodium hydroxide solution in conventional chemical deinking.

To the enzyme component system (ECS) are added besides the usual components such as lipase, oxidant, fatty acid and ketone, also a number of phenolic substances which serve as polymerization catalysts in wastewater treatment and in lignin
20 polymerization/modification applications. Here, we found, surprisingly, that these substances cause a shift in the pH for the optimum enzyme activity thus improving performance.

Also surprisingly, we found that the addition of a reducing agent, preferably dithionite or bisulfite increases the efficiency of ink detachment.

25 One component of the enzyme component system (ECS) of the invention used is an enzyme, preferably lipase from *Humicola lanuginosa*, at a concentration from 5 to 500 mg per kg of air-dried waste paper, and preferably from 5 to 100 mg of enzyme per kg of waste paper (corresponding to about 25,000 to 500,000 IU per kg of waste paper) (1 IU hydrolyzes 1 μ equivalent of triglyceride fatty acid in 1 hour at PH 7.7 and 37
30 $^{\circ}\text{C}$). The treatment of the waste paper with the enzyme component system of the invention for the purpose of removing printing ink particles is preferably carried out in the presence of oxygen or air at atmospheric pressure or slightly positive oxygen

pressure (maximum 2 bar) and at a pH from 7 to 11 and preferably 7 to 9, at a temperature from 20 to 95 °C and preferably from 40 to 95 °C.

Another component is the oxidant, preferably H₂O₂, which is used at a concentration from 5 to 5000 mg per kg of waste paper (100% basis) and preferably from 5 to 1000 mg per kg of waste paper.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆, and particularly C₆ to C₁₆, fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 5 to 2000 mg per kg of waste paper and preferably at a concentration from 5 to 500 mg per kg of waste paper.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 5 to 2000 mg per kg of waste paper and preferably at a concentration from 5 to 500 mg per kg of waste paper.

Moreover, to increase the efficiency of the process, the abovesaid compounds are used, for example phenolic substances or polycyclic compounds with several oxidizable

hydroxyl groups and preferably, for example, bisphenol A. These substances are employed at a concentration from 1 to 2000 mg per kg of waste paper and preferably at a concentration from 1 to 500 mg per kg of waste paper.

In addition, a reducing agent is used, preferably Na-dithionite or Na-bisulfite, at a concentration from 0. 1 to 1000 mg per kg of waste paper and preferably at a

concentration from 0. 1 to 200 mg per kg of waste paper.

To collect the printing ink particles, commercial detergents are used as collectors, preferably Incopur brands, for example Incopur RSGA, at a concentration from 1 to 5000 mg per kg of waste paper, and preferably from 1 to 1000 mg per kg of waste paper.

To enhance the detaching effect on many waste paper compositions, additional enzymes can be added, for example cellulases and/or hemicellulases (for example, xylanase and/or mannase etc.) and/or pectinases and/or oxidoreductases.

The invention will be further illustrated by way of the following examples:

EXAMPLE 7

About 10 kg of water (preheated to about 45 °C) was added to the pulper of a Lamort laboratory deinking apparatus, and the pH was adjusted with sodium hydroxide solution (and/or sulfuric acid) so that after the addition of 1.5 kg of air-dried waste paper (50% newspapers, 50% magazines) which had been cut into about 2 x 3 cm pieces and after

5 the addition of the other system constituents the pH was 8.0 to 8.5.

These system constituents were (per kg of air-dried waste paper):

a) 500,000 IU of lipase from *Humicola lanuginosa* per 100 mL of tap water

b) 0.1 g of dodecanoic acid per 100 mL of tap water

c) 0.1 g of benzophenone per 100 mL of tap water

10 d) 0.1 g of bisphenol A per 20 mL of 0.1 molar NaOH

e) 0.02 g of Na bisulfite per 10 mL of tap water

f) 0.5 g of Incopur RSGA per 100 mL of tap water

g) 1 g of H₂O₂ (30%) per 100 mL of tap water (added at the end).

15 The pulper was started after the addition of system constituents a) to g) and during the addition of the waste paper. The total quantity of water was then adjusted to 15 kg with approximately 45 °C tap water. The pulping process was allowed to proceed for 10 minutes. For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40-45 °C for 15 to 45 minutes.

20 In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness were determined. Table 3 shows the results.

25

(ISO = International Standardization Organization)

EXAMPLE 8

30

The method was the same as in Example 7, but instead of the enzyme component system (ECS) of the invention only water was used. Table 3 shows the results.

EXAMPLE 9

About 1 kg of water (preheated to about 45 °C) was added to a dough mixer, and the pH was adjusted with sodium hydroxide solution (and/or sulfuric acid) so that after the addition of 150 g of air-dried waste paper (50% newspapers, 50% magazines) which had been cut into about 2 x 3 cm pieces and after the addition of the other system constituents the pH was 8.0 to 8.5.

These system constituents were (per 100 g of air-dried waste paper):

10 a) 5000 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691) per 100 mL of tap water

(1 IU = conversion of 1 μ mole of acetamide and hydroxylamine to acetohydroxamic acid and NH_3 per minute at pH 7.2 and 37 °C)

b) 0.01 g of dodecanoic acid per 100 mL of tap water

15 c) 0.01 g of benzophenone per 100 mL of tap water

d) 0.01 g of bisphenol A per 20 mL of 0.1 molar NaOH

e) 0.002 g of Na bisulfite per 10 mL of tap water

f) 0.05 g of Incopur RSGA per 100 mL of tap water

g) 0.1 g of H_2O_2 (30%) per 100 mL of tap water (added at the end).

20 The dough mixer was started after the addition of system constituents a) to g) and during the addition of the waste paper. The total quantity of water was then adjusted to 1.5 kg with tap water (45 °C). The pulping process was allowed to proceed for 10 minutes.

25 For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40-45 °C for 15 to 45 minutes.

In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were 30 prepared from it in a commercial sheet former, dried and the ISO brightness and were determined. Table 3 shows the results.

EXAMPLE 10 (Chemical System)

About 10 kg of water (preheated to about 45 °C) was added to the pulper of a Lamort

5 laboratory deinking apparatus, and 1.5 kg of air-dried waste paper (50% newspapers, 50% magazines), cut into approximately 2 x 3 cm pieces, was added after the addition of the following chemicals (based on air- dried pulp):

- 1) 0.8 wt % of soap (DR 3, Henkel)
- 10 2) 3.5% of water glass
- 3) 2% of sodium hydroxide (100%)
- 4) 1 % H_2O_2 (100%)

The pulper was started during waste paper addition. The total quantity of water was then

15 adjusted to 15 kg with about 45 °C tap water. The pulping process was allowed to proceed for 10 minutes.

For further reaction, the fiber suspension was transferred to a heated vessel where it was allowed to incubate at about 40-45 °C for 15 to 45 minutes,

20 In a Voith flotation cell, 100 g of pulp, absolutely dry basis, (after this incubation) was adjusted to a total volume of about 20 L with tap water (45 °C) and subjected to flotation for 10 to 20 minutes. The accepted stock was discharged, test sheets were prepared from it in a commercial sheet former, dried and the ISO brightness were determined'. Table 3 shows the results.

25

30

35

TABLE 3

System	ISO Brightness, %
5 Water only	51
Chemical system	61
ECS/lipase	58.5
ECS/amidase	57
10 Comparative system: laccase (800,000 IU/kg of waste paper + bisphenol A + Na bisulfite (0.1 or 0.02 g/kg of waste paper), for other con- ditions see WO 91/14820; WO 92/20857	55.5
15	

V) Use as Oxidation System in Organic Synthesis

20 From the multiplicity of possible uses of the enzyme component system (ECS) of the invention, such as in hydroxylation reactions, oxidation of unsaturated aliphatics, Baeyer-Villiger oxidations, oxidation of heterocycles, carbon-carbon dehydrogenations and other oxidation reactions, by way of the following examples, the oxidation of 25 alcohols to aldehydes and of aromatic methyl groups to aldehydes are described.

It is known from the literature that these reactions can be carried out with the 30 oxidoreductase laccase and a mediator such as ABTS (2,2'-azino-bis(3-ethylbenzothiazolin-6-sulfonic acid), see T. Rosenau et al., Synthetic Communications 26 (2), 315-320 (1996) and A Potthast et al., J. Org. Chem. (60), pp. 4320-4321 (1995). The main advantage of the process of the invention over these processes is its lower cost and better performance, particularly based on the cost. One of the components of the enzyme component system (ECS) of the invention is an 35 enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a concentration of 0.05 to 5 mg per 10 mmoles of substrate, preferably from 0.05 to 3 mg per 10 mmoles of substrate (which corresponds to about 250 to 15,000 IU) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

Preferably, the oxidation reaction with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or a slightly positive O_2 pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a 5 to 100 mmolar, preferably 5 to 50 mmolar, substrate concentration.

Another component is the oxidant, preferably H_2O_2 (100%), which is used at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably from 0.05 to 30 mg per 10 mmoles of substrate.

Another component consists of one or more fatty acids, preferably C_6 to C_{26} , and particularly C_6 to C_{16} fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably at a concentration from 0.05 to 30 mg per 10 mmoles of substrate.

Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 100 mg per 10 mmoles of substrate and preferably at a concentration from 0.05 to 30 mg per 10 mmoles of substrate.

The invention will be further illustrated by way of the following examples:

EXAMPLE 11 (Oxidation of Benzyl Alcohols to Aldehydes)

The following components were added to 50 mL of 0.1 molar pH 4.5 acetate buffer in a 250-mL reaction flask:

- 1) p-methoxybenzyl alcohol in 30 mL of tetrahydrofuran [THF] (20-molar in total volume)
- 2) 2 mg of lipase from *Humicola lanuginosa*
- 3) 5 mg of dodecanoic acid
- 4) 25 mg of benzophenone

The reaction was started by addition of 12.5 mg of H_2O_2 (30%) and was allowed to proceed for 12 to 24 hours. Then, 0.5 mL of the reaction solution was removed, extracted with CH_2Cl_2 , and the p-methoxybenzaldehyde content was determined by GC or GC-MS. The results are shown in Table 4.

EXAMPLE 12 (Oxidation of Aromatic Methyl Groups to Aldehydes)

5 The following components were added to 50 mL of 0.1 molar pH 4.5 acetate buffer in a
250-mL reaction flask:

- 1) toluene in 30 mL of THF (20-molar in total volume)
- 2) 2 mg of lipase from *Humicola lanuginosa*
- 3) 5 mg of dodecanoic acid
- 10 4) 25 mg of benzophenone

The reaction was started by addition of 12.5 mg of H_2O_2 (30%) and was allowed to proceed for 12 to 24 hours. Then, 0.5 mL of the reaction solution was removed, extracted with CH_2Cl_2 , and the benzaldehyde content was determined by GC or GC-
15 MS. The results are shown in Table 4.

TABLE 4

	Substrate	Oxidized Substrate	Conversion, %
20	p-Methoxybenzyl alcohol (lipase)	p-methoxybenzaldehyde	98
25	p-Methoxybenzyl alcohol (ABTS/laccase)	p-methoxybenzaldehyde	90
30	Toluene (lipase)	benzaldehyde	98
35	Toluene (ABTS/laccase)	benzaldehyde	92

VI) Use in Coal Liquefaction

Recently, the use of white rotting fungi was studied in the liquefaction of lignite and anthracite, and the general feasibility thereof was confirmed. Patent applications WO 94/29510 and WO 96/18770 have also disclosed the general feasibility of using fungus-free systems based on oxido- reductases and special mediators.

5 Surprisingly, we were able to confirm for the enzyme component system (ECS) of the invention that it, too, can be used for "liquefying" the lignin-like tridimensional network of polycyclic aromatic ring systems of lignite and anthracite.

One of the components of the enzyme component system (ECS) of the invention is an enzyme, preferably lipase from, for example, *Humicola lanuginosa*, used at a

10 concentration of 0.05 to 20 mg per gram of ground lignite, absolutely dry basis, preferably from 0.05 to 10 mg per gram of coal (corresponding to about 250 to 50,000 IU) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C).

15 Preferably, the coal treatment with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive O₂ pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C, and a coal consistency of 0.5 to 40%.

20 An unusual and surprising finding concerning the use of enzymes is that when the enzyme component system of the invention is employed, the consistency of the material can be increased and the performance is markedly improved. For economic reasons, the process according to the invention is carried out at a coal consistency from 4 to 35% and particularly from 4 to 15%.

25 Another component is the oxidant, preferably H₂O₂, which is used at a concentration from 0.05 to 100 mg per gram of coal (100% basis) and preferably from 0.05 to 50 mg per gram of coal.

Another component consists of one or more fatty acids, preferably C₆ to C₂₆, particularly C₆ to C₁₆ fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 100 mg per gram of coal and preferably at a concentration from 0.05 to 50 mg per gram of coal.

30 Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 100 mg per gram of coal and preferably at a concentration from 0.05 to 50 mg per gram of coal.

The invention will be further illustrated by way of the following example:

EXAMPLE 13

5 Enzymatic Coal Liquefaction

5 g of lignite or anthracite, absolutely dry basis, (particle size about 200 to 500, μ) was added to solutions prepared as follows:

- 10 A) To 20 mL of tap water were added 5 mg of tetradecanoic acid, 25 mg of benzophenone and 12.5 mg of H₂O₂ (30%) per gram of coal with agitation. The pH was adjusted with sulfuric acid and/or sodium hydroxide solution so that, after addition of the coal and the enzyme, the pH was 8.
- B) To 5 mL of tap water was added 10 mg of lipase from *Humicola lanuginosa* (about 15 50,000 IU).

Solutions A and B were combined and diluted to 45 mL. After addition of the coal, the material was mixed for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours.

The resulting coal of modified consistency was then removed from the reaction flask.

20

VII) Use as Bleaching Agent in Detergents

In using the enzyme component System (ECS) of the invention as a bleaching agent in detergents, one of the components is an enzyme, preferably lipase from, for example,

- 25 *Humicola lanuginosa*, used at a concentration from 0.05 to 20 mg per 100 mL of washing solution and preferably from 0.05 to 10 mg of enzyme per 100 mL of washing solution (which corresponds to about 250 to 100,000 IU per 100 mL of washing solution) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at PH 7.7 and 37 °C).

- 30 Preferably, the bleaching with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a slightly positive O₂

pressure and at pH from 2 to 12, preferably at pH 3-10, at a temperature of 20 to 95 °C and preferably 30-95 °C.

Another component is the oxidant, preferably H₂O₂, which is used at a concentration from 0.05 to 50 mg per 100 mL of washing solution (100% basis) and preferably from

5 0.05 to 20 mg per 100 mL of washing solution.

Another component consists of one or more fatty acids, preferably a C₆ to C₂₆, particularly a C₆ to C₁₆ fatty acid and more particularly tetradecanoic or dodecanoic acid, at a concentration from 0.05 to 50 mg per 100 mL of washing solution and preferably at a concentration from 0.05 to 20 mg per 100 mL of washing solution.

10 Another component is a ketone, preferably, for example, benzophenone at a concentration from 0.05 to 50 mg per 100 mL of washing solution and preferably at a concentration from 0.05 to 20 mg per 100 mL of washing solution.

Other Components

15 The bleaching system can also contain phenolic and/or nonphenolic compounds with one or more benzene rings. The following oxidants besides those mentioned hereinabove are particularly preferred: air, oxygen, H₂O₂, organic peroxides, sodium perborate and/or sodium percarbonate. Oxygen can also be generated in situ by H₂O₂+

20 catalase or the like, or H₂O₂ can be generated in situ by GOD + glucose or similar systems.

Also preferred are multicomponent bleaching systems containing cation-generating metals salts. The cations Fe²⁺, Fe³⁺, Mn²⁺, Mn³⁺, Mn⁴⁺, Cu¹⁺, Cu²⁺, Ti³⁺, Ce⁴⁺, Mg²⁺ and Al³⁺ are preferably used.

25 The bleaching system can also contain polysaccharides and/or proteins. Suitable polysaccharides are, glucans, mannans, dextrans, levans, pectins, alginates, vegetable gums and/or polysaccharides formed by fungi or produced in mixed cultures with yeasts. Suitable proteins are gelatins and albumins, among others. Also suitable are monosaccharides, oligosaccharides, amino acids, PEG, polyethylene oxides, 30 polyethylenimines and polydimethylsiloxanes.

Use of the Multicomponent System

The multicomponent system can be used in combination with surface-active detergent constituents or detergent additives, which in themselves are known.

5 The invention will be further illustrated by way of the following examples:

EXAMPLE 14

Effect of ECS on Tea-Stained Standard Cotton Fabrics

10 A (5x5 cm) piece of fabric was allowed to incubate in 100 mL of washing solution (in a 300-mL Erlenmeyer flask) at 40 °C for 40 min with reciprocating shaking (120 rpm). Before the beginning of incubation, the washing solution was subjected to a 10-min temperature equilibration period.

15 The washing solution was prepared with standard tap water (STW) at 14° dH. The following system component doses were used: 2.5 mg of lipase from *Humicola lanuginosa*/100 mL, 2.5 mg of tetradecanoic acid/100 mL, 12.5 mg of benzophenone/100 mL and 6.5 mg of H₂O₂ (30%). After decanting the washing liquor", cold water was added 3x in the form of a strong water jet and then decanted.

20 The results are shown in Table 5.

EXAMPLE 15

Effect of ECS (Amidase) on Tea-Stained Standard Cotton Fabrics

25 A (5x5 cm) piece of fabric was allowed to incubate in 100 mL of washing solution (in a 300-mL Erlenmeyer flask) at 40 °C for 40 min with reciprocating shaking (120 rpm). Before the beginning of incubation, the washing solution was subjected to a 10-min temperature equilibration period.

30 The washing solution was prepared with standard tap water (STW) at 14° dH. The following doses were used: 1000 IU of amidase/ 100 mL (1 IU = conversion of 1 μmole of acetamide and hydroxylamine to acetohydroxamic acid per minute at pH 7.2 and 37 °

C), 2.5 mg of tetradecanoic acid/100 mL, 12.5 mg of benzophenone/100 mL and 6.5 mg of H₂O₂ (30%). After decanting the "washing liquor", cold water was added 3 x in the form of a strong water jet and then decanted.

The results are shown in Table 5.

5 (dH = one degree of German water hardness = 10 mg of CaO/L)

TABLE 5

		pH	Whiteness	Brightness
10	STW zero value	4.5	2.55	2.3
	Heavy-duty detergent	10.1	8.9	6.15
	STW + ECS (lipase)	8.5	7.5	7.2
15	STW + ECS (amidase)	8	6.9	6.3
	Liquid detergent + ECS (lipase)	8.5	8.5	8.0
	Comparative test: Liquid detergent + laccase			
20	+ HOBT (conditions as in PCT/EP 96/02658; PCT/EP 94101967	5	6.5	6.0

25

VIII) Use in the Bleaching/Decolorizing of Textiles

One of the components of the enzyme component system (ECS) of the invention used in the bleaching/decolorizing of textile fabrics is an enzyme, preferably lipase from, for

30 example, *Humicola lanuginosa*, employed at a concentration of 0.05 to 10 mg per gram of denim (corresponding to about 250 to 25,000 IU per gram of denim) (1 IU hydrolyzes 1 μ equivalent of a triglyceride fatty acid in 1 hour at pH 7.7 and 37 °C). Preferably, the bleaching/decolorizing with the enzyme component system of the invention is carried out in the presence of oxygen or air at atmospheric pressure or at a 35 slightly positive O₂ pressure and at pH from 2 to 11, preferably at pH 3-9, at a temperature of 20 to 95 °C, preferably 40-95 °C and a fabric density of 0.5 to 40% [fabric density = ratio of weight of fabric to weight of solution]

An unusual and surprising finding concerning the use of enzymes is that when the enzyme component system of the invention is used, the fabric density can be increased and the performance markedly improved. For economic reasons, the process according to the invention is carried out at a fabric density from 4 to 35% and particularly from 4 to 15%.

5 Another component is the oxidant, preferably H_2O_2 , which is used at a concentration from 0.05 to 20 mg per gram of denim (100% basis) and preferably from 0.05 to 10 mg per gram of denim.

10 Another component consists of one or more fatty acids, preferably C_6 to C_{26} and particularly C_6 to C_{16} fatty acids, and more particularly tetradecanoic or dodecanoic acid at a concentration from 0.05 to 20 mg/g of denim and preferably at a concentration from 0.05 to 10 mg per gram of denim.

15 Another component is a ketone, preferably, for example, benzophenone, used at a concentration from 0.05 to 20 mg/g of denim and preferably at a concentration from 0.05 to 10 mg/g of denim.

The invention will be further illustrated by way of the following examples:

EXAMPLE 16

20 Bleaching with ECS + Lipase

1 g of denim fabric was placed in a 200-mL Erlenmeyer flask (fabric density 2%). The pH of the solution (tap water), the volume of which was 50 mL after the addition of all components, was preadjusted to pH 6 with 0.5 N H_2SO_4 .

25 1 mg of lipase from *Humicola lanuginosa*, 0.5 mg of tetradecanoic acid, 1 mg of benzophenone and 2.5 mg of H_2O_2 (30%) were added per gram of denim. The experiment was carried out in a shaking water bath (200 rpm) at 45 °C and a reaction time of 45 minutes. The piece of fabric was washed with tap water and dried in air. The brightness was then determined with an Elrepho instrument. The values obtained are 30 given in Table 6.

EXAMPLE 17**Bleaching with ECS + Amidase**

5 1 g of denim fabric was placed in a 200-mL Erlenmeyer flask (fabric density 2%). The pH of the solution (tap water), the volume of which was 50 mL after the addition of all components, was preadjusted to pH 6 with 0.5 N H₂SO₄.

10 200 IU of amidase from *Pseudomonas aeruginosa* (Sigma A 6691), 0.5 mg of tetradecanoic acid, 1 mg of benzophenone and 2.5 mg of H₂O₂ (30%) were added per gram of denim. The experiment was carried out in a shaking water bath (200 rpm) at 45 °C and a reaction time of 45 minutes. The piece of fabric was washed with tap water and dried in air. The brightness was then determined with an Elrepho instrument. The values obtained are given in Table 6.

TABLE 6

System	pH	ISO Brightness
Untreated specimen	--	4.5
Laccase + violuric acid (comparative system)	3.5	13.5
ECS system + lipase	6.0	16.9
ECS system + amidase	6.0	14.5
Hypochlorite	4.5	n.d.

Addition of Other Substances to the Enzyme Component System (ECS)

30 For all applications, the components of the enzymatic oxidation systems with enzyme action-enhancing compounds disclosed in DE 198 21 263.1, DE 198 20 947.9 and PCT/DE 98/01313 can be added to the enzyme component system (ECS) of the invention, such systems containing the following:

35 a) At least one oxidation catalyst, preferably enzymes such as oxidoreductases of classes 1.1.1. to 1.97 according to the International Enzyme Nomenclature: Committee of the International Union of Biochemistry and Molecular Biology (Enzyme

Nomenclature, Academic Press, Inc., 1992, pp. 24-154) among which the following are particularly preferred: cellobiose: oxygen-1-oxidoreductase (cellobiose reductase)

1.1.3.25, cellobiose: quinone-l-oxidoreductase **1.1.5.1**, bilirubin oxidase **1.3.3.5**, cytochrome oxidase **1.9.3**, oxygenases, lipoxygenases **1.13**, **1.14**, superoxide dismutase

5 **1.15.11**, ferrioxidase, for example ceruloplasmin **1.16.3.1**, especially preferred being the enzymes of class **1.10** which act on related compounds. They catalyze the oxidation of biphenols and ascorbates. Suitable acceptors are NAD⁺, NADP⁺ (**1.10.1**), cytochrome (**1.10.2**), oxygen (**1.10.3**) or others (**1.10.99**). Among these, particularly preferred as acceptors are the enzymes of class **1.10.3** with oxygen (O₂) as acceptor.

10 Particularly preferred among the enzymes of this class are catechol oxidase (tyrosinase) (**1.10.3.1**), L- ascorbate oxidase (**1.10.3.3**), O-aminophenol oxidase (**1.10.3.4**) and laccase (benzenediol: oxygen oxidoreductase) (**1.10.3.2**), the laccases (benzenediol: oxygen oxidoreductase) (**1.10.3.2**) being particularly preferred.

15 Other particularly preferred enzymes are those of group **1.11** which act on a peroxide as acceptor. Only subclass (**1.11.1**) contains peroxidases. Especially preferred here are cytochrome C peroxidases (**1.11.1.5**), catalase (**1.11.1.6**), peroxidase (**1.11.1.7**), iodide peroxidase, (**1.11.1.8**), glutathione peroxidase (**1.11.1.9**), chloride peroxidase (**1.11.1.10**), L-ascorbate peroxidase (**1.11.1.11**), phospholipid hydroperoxide glutathione peroxidase (**1.11.1.12**), manganese peroxidase (**1.11.1.13**) and diarylpropane peroxidase (ligninase, lignin peroxidase) (**1.11.1.14**).

b) At least one suitable oxidant,

25 c) At least one mediator selected from the group consisting of hydroxylamines, hydroxylamine derivatives, hydroxamic acids, hydroxamic acid derivatives, aliphatic, cycloaliphatic, heterocyclic or aromatic compounds containing at least one N-hydroxy, oxime, N-oxy or N,N'-dioxy function and/or at least one mediator from the group of amides, such as, for example, hydrazides or 1,2,4-triazolidin- 3,5-diones (urazoles) and/or at least one mediator from the group of imides such as, for example, the hydantoins, and/or at least one mediator from the group of oxocarbons.

Moreover, it is possible to use at least one **mediation enhancer** selected from the group consisting of carbonyl compounds, aliphatic ethers, phenol ethers or olefins (alkenes), and/or at least one **mediation enhancer** selected from the group consisting of the abovesaid mediators of the NO-, NOH- or HRN-OH type and/or amides such as the 5 hydrazides or urazoles and/or the imides such as the hydantoins and/or the oxocarbons.

It is also possible to use at least one mediation enhancer selected from the group consisting of cation radical-generating substances of the phenothiazine and/or phenoxazine type and/or of the (R = N-N = R) type* (for example, ABTS) or of aryl-substituted alcohols (nonphenols) such as, for example, veratryl alcohol and/or phenol derivatives, such as p-hydroxycinnamic acid, 2,4-dichlorophenol, p-10 hydroxybenzenesulfonate, vanillin (4-hydroxy-3-methoxybenzaldehyde), p-hydroxybenzoic acid, 5- amino-2-hydroxybenzoic acid (5-aminosalycilic acid) and/or Wurster-type radical cation compounds [see Angewandte Chemie 91, pp. 982-997 (1979); Chem. Unserer Zeit 12, pp. 89-98 (1978); Römpf Chemie Lexikon [Römpf Chemical Encyclopedial, 9th edition, 1995] and/or radical anions, for example 15 semiquinones formed by enzymatic oxidation of hydroquinones.

It is essential for improving the performance of the enzyme/mediator systems with the aid of comediators that the mediator/comediator ratio be from 5000 : 1 to 1 : 1, a ratio 20 from 500 : 1 to 1 : 1 being particularly preferred. When several mediators and comediators are used at the same time, the ratio of these mediator or comediator concentrations depends on the particular combinations employed.

* N means nitrogen, R denotes groups.

25

These enzymatic oxidation systems according to the invention contain at least one 30 oxidant. Suitable oxidants are, for example, air, oxygen, ozone, peroxides, such as H₂O₂, organic peroxides, per acids such as peracetic, performic, persulfuric, pernitric, metachloroperoxybenzoic and perchloric acid, per compounds such as perborates, percarbonates or persulfates, or oxygen species and the radicals thereof such as the OH,

OOH and OH⁺ radical, superoxide (O₂⁻), dioxygenyl cation O₂⁺, singlet oxygen, ozonide (O₃[·]), dioxiranes, dioxitanes or Fremy radicals.

5 Said preferred mediator/mediation enhancer substances of formulas I to XXII and the said other mediation-enhancing compounds are represented in Appendix IV and Appendix IVa.

10 In the following, by way of an example of enzymatic pulp bleaching, the improvement in performance is presented that can be achieved for many types of pulps by use of a combination of the enzyme component system (ECS) of the invention and the above-described oxidation systems with enzyme action-enhancing compounds.

EXAMPLE 18 (Enzyme: lipase/laccase)

15 **Enzymatic Bleaching of Softwood (Sulfate Pulp)**

5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

20 A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone, 2.5 mg of H₂O₂ (30%), 37 µmoles of violuric acid + 0.37 µmole of 4-tert.butylurazole with agitation. The pH was adjusted with 0.5 mole/L sulfuric acid solution so that, after addition of the wood pulp and the enzyme, the pH was 5.

25 B) To 5 mL of lap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU), and to this mixture was added an amount of laccase from *Trametes versicolor* sufficient to give an activity of 15 U (1 U = conversion of 1 µmole of ABTS/min/mL of enzyme) per gram of wood pulp.

Solutions A and B were combined and diluted to 33 mL. After addition of the wood 30 pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours at atmospheric pressure.

The pulp was washed over a nylon screen (30 μ m) and extracted for one hour at 60 °C, 2% pulp consistency and using 8% NaOH per gram of wood pulp. The pulp was again washed, after which the kappa number was determined. For results see Table 7.

5

EXAMPLE 19 (Enzyme: lipase/peroxidase)**Enzymatic Bleaching of Softwood (Sulfate Pulp)**

10 5 g, absolutely dry basis, of wood pulp (O₂-delignified softwood), pulp consistency 30% (about 17 g moist) was added to solutions prepared as follows:

A) To 20 mL of tap water were added 1 mg of tetradecanoic acid, 5 mg of benzophenone, 2.5 mg of H₂O₂ (30%), 37 μ moles of violuric acid + 0.37 μ mole of 4-

15 tert.butylurazole with agitation. The pH was adjusted with 0.5 mole/L sulfuric acid solution so that, after addition of the wood pulp and the enzyme, the pH was 7.

B) To 5 mL of tap water was added 5 mg of lipase from *Humicola lanuginosa* (about 25,000 IU) and 0.1 mg of peroxidase (horseradish) per gram of wood pulp.

20 Solutions A and B were combined and diluted to 33 mL. After addition of the wood pulp, the material was mixed in a dough mixer for 2 minutes. The material was then transferred to a reaction vessel preheated to 45 °C and allowed to incubate 1-4 hours at atmospheric pressure.

The pulp was washed over a nylon screen (30 μ m) and extracted for one hour at 60 °C, 25 2% pulp consistency and using 8% NaOH per gram of wood pulp. The pulp was again washed, after which the kappa number was determined. For results see Table 7.

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TABLE 7**SYSTEM****% DELIG.**
(lipase/lacc.)**% DELIG.**
(lipase/peroxid.)

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ECS + lipase system
(+ laccase + violuric acid) +*

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ECS + lipase system
(+ peroxidase + violuric acid) +*

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Laccase (+ violuric acid) +*

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Peroxidase (+ violuric acid) +*

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* **Mediation enhancer** 4-tert butylurazole

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APPENDIX I**System Component 2 of the Enzyme Component System (ECS) of the Invention**

5 The fatty acids used in the process of the invention as a source of peracid are, for example:

1) Saturated fatty acids

Butanoic acid	(butyric acid)
10 Pentanoic acid	(valeric acid)
Hexanoic acid	(caproic acid)
Heptanoic acid	(enanthic acid)
Octanoic acid	(caprylic acid)
Nonanoic acid	(pelargonic acid)
15 Decanoic acid	(capric acid)
Undecanoic acid	
Dodecanoic acid	(lauric acid)
Tridecanoic acid	
Tetradecanoic acid	(myristic acid)
20 Pentadecanoic acid	
Hexadecanoic acid	(palmitic acid)
Heptadecanoic acid	
Octadecanoic acid	(stearic acid)
Nonadecanoic acid	
25 Eicosanoic acid	(arachic acid)
Heneicosanoic acid	
Docosanoic acid	(behenic acid)
Tricosanoic acid	
Tetracosanoic acid	(lignoceric acid)
30 Pentacosanoic acid	
Hexacosanoic acid	(cerotic acid)
Octacosanoic acid	
Triacontanoic acid	(melissic acid)

2) Unsaturated fatty acids

	10-Undecenoic acid	
	9-cis-Dodecenoic acid	(lauroleic acid)
5	9-cis-Tetradecenoic acid	(myristoleic acid)
	9-cis-Hexadecenoic acid	(paimitoleic acid)
	6-cis-Octadecenoic acid	(petroselic acid)
	6-trans-Octadecenoic acid	(petroselaidic acid)
	9-cis-Octadecenoic acid	(oleic acid)
10	9-trans-Octadecenoic acid	(elaidic acid)
	9-cis, 12 cis-Octadecadienoic acid	(linoleic acid)
	9-trans, 1 2-trans-Octadecadienoic acid	(linolaidic acid)
	9-cis, 12-cis,15-cis-Octadecatrienoic acid	(linolenic acid)
	9-trans, 11 -trans, 1 3-trans- Octadecatrienoic acid	(α -eleostearic acid)
15	9-cis, 11 -trans, 1 3-trans-Octadecatrienoic acid	(β -eleostearic acid)
	9-cis-Icosenic acid	(gadoleic acid)
	Icosa-5,8,11,14-tetraenoic acid	(arachidic acid)
	13-cis-Docosenoic acid	(erucic acid)
	13-trans-Docosenoic acid	(brassidic acid)
20	4,8,12,15,19-Docosapentaenoic acid	(clupanodonic acid)

3) Polyunsaturated fatty acids

25	9,12-Octadecadienoic acid	(linoleic acid)
	9,12,1 5-Octadecatrienoic acid	(linolenic acid)
	5,9,1 2-Octadecatrienoic acid	
	9,11,13-Octadecatrienoic acid	(eleostearic acid)
	9,11,13,15-Octadecatetraenoic acid	(parinaric acid)
30	5,11,14-Icosatrienoic acid	
	5,8,11,1 4-Icosatetraenoic acid	(arachidic acid)
	4,8,12,15,1 8-Icosapentaenoic acid	

4,8,12,15,19-Docosapentaenoic acid	(clupanodonic acid)
4,8,12,15,18,21 -Tetracosahexaenoic acid	(nisinic acid)

Particularly preferred are tetradecanoic acid (myristic acid) and dodecanoic acid (lauric acid).

APPENDIX II

System Component 4 (Ketones) of the Enzyme Component System (ECS) of the Invention

Particularly preferred are carbonyl compounds of general formula I:

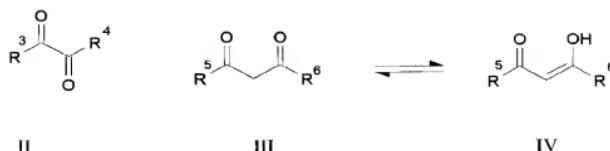


The R^1 and R^2 groups can be equal or different and denote aliphatic or aromatic groups. Moreover, the R^1 and R^2 groups can form a ring containing besides carbon also

20 heteroatoms such as nitrogen, oxygen and sulfur.

Particularly preferred are 1,2-diketones (formula II), 1,3-diketones (formula III), polyketones (polyketides) and the tautomeric enols (formula IV):

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wherein the R³ to R⁶ groups, once again, can be equal or different and denote aliphatic or aromatic groups. Moreover, groups R³ and R⁴ and groups R⁵ and R⁶, together, can form a ring containing besides carbon also heteroatoms such as nitrogen, oxygen or sulfur. The possibility of tautomerization or formation of a resonance hybrid is particularly important in this case.

Besides general carbonyl compounds, particularly preferred are ketones, such as, in general hydroxyketones, α,β -unsaturated ketones, oxycarboxylic acids, quinones and halogenated ketones.

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Particularly preferred among these are the following:

Acetone, methyl ethyl ketone, diethyl ketone, methyl n-butyl ketone, methyl isobutyl ketone, cyclohexanone, cyclopentanone, 2-methylcyclohexanone, 3-

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methylcyclohexanone, 4-methylcyclohexanone, dihydroxyacetone, diacetyl monohydrazone, diacetyl dihydrazone, acetophenone, p-hydroxyacetophenone, 1-phenyl-3-butanone, 3-pentanone, 4-heptanone, 2-nonanone, cycloheptanone, cyclooctanone, cyclodecanone, cyclododecanone, dimethyl ketone, ethyl propyl ketone, methyl amyl ketone, acetylacetone, pinacoline, methyl isopropyl ketone, methyl

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isoamyl ketone, ethyl amyl ketone diisopropyl ketone, diisobutyl ketone, methyl vinyl ketone, methyl isopropenyl ketone, mesityl oxide, isophorone, hydroxyacetone, methoxyacetone, 2,3-pentanedione, 2,3-hexanedione, phenylacetone, propiophenone, benzophenone, benzoin, benzil, 4,4'-dimethoxybenzil, 4'-methoxyacetophenone, 3'-methoxyacetophenone, O-ethylbenzoin, (2-methoxyphenyl)acetone, (4-

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methoxyphenyl)acetone, methoxy-2-propanone, glyoxylic acid, benzyl glyoxylate,

benzylacetone, methyl benzyl ketone, methylcyclohexyl ketone, 2-decanone,

dicyclohexyl ketone, 3,3-dimethyl-2-butanone, methyl isobutyl ketone, methyl

isopropyl ketone, 2-methyl-3-heptanone, 5-methyl-3-heptanone, 6-methyl-5-hepten-2-one, 5-methyl-2-hexanone, 3-nonanone, 5-nonanone, 2-octanone, 3-octanone, 2-

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undecanone, 1,3-dichloroacetone, 1-hydroxy-2-butanone, 3-hydroxy-2-butanone, 4-

hydroxy-4-methyl-2-pentanone, 2-(1S)-adamantanone, anthrone, bicyclo(3.2.0)hept-2-

en-6-one, cis-bicyclo(3.3.0)octan-3,7-dione, (1S)-(-)-camphor, p-chloranil,

cyclobutanone, 1,3-cyclohexanedione, 1,4-cyclohexanedione monoethylene ketal,
5 dibenzosuberone, ethyl 4-oxocyclohexanecarboxylate, 9-fluorenone, 1,3-indandione,
methyl- cyclohexanone, phenylcyclohexanone, 4-propylcyclohexanone, 1,2,3,4-
tetrahydro-1-naphthalenone, 1,2,3,4-tetrahydro-2-naphthalenone, 3,3,5-trimethylcyclo-
hexanone, 3-acetoxy-2-cyclohexen-1-one, benzylideneacetone, (R)-(-)-carvone,
(S)-(-)-carvone, curcumin, 2-cyclohexen-1-one, 2,3-diphenyl-2- cyclopropen-1-one, 2-
hydroxy-3-methyl-2-cyclopentene-1-one, isophorone, α -ionone, β -ionone, 3-methoxy-2-
10 cyclohexen-1-one, 3-methyl-2-cyclopenten-1-one, 3-methyl-3-penten-2-one, (R)-(+)-
pulegone, tetraphenyl-2,4-cyclopentadien-1-one, 2,6,6-trimethyl-2-cyclohexen-1,4-
dione, 2-acetylbenzoic acid, 1-acetyl naphthalene, 2-acetyl naphthalene,
3'-aminoacetophenone, 4'-aminoacetophenone, 4'-cyclohexylacetophenone, 3',4'-
15 diacetoxyacetophenone, diacetylbenzene, 2',4'-dihydroxyacetophenone, 2',5'-
dihydroxyacetophenone, 2',6'-dihydroxyacetophenone, 3,4-dimethoxyacetophenone,
2'-hydroxyacetophenone, 4'-hydroxyacetophenone, 3'-methoxyacetophenone, 4'-
methoxyacetophenone, 2'-methylacetophenone, 4'-methylacetophenone,
2'-nitroacetophenone, 3'-nitroacetophenone, 4'-phenylacetophenone, 3',4',5'-trimethoxy-
acetophenone, 4'-aminopropiophenone, benzoylacetone, benzoylpropionic acid,
benzylideneacetophenone, cyclohexyl phenyl ketone, desoxybenzoin, 4',4'-
20 dimethoxybenzil, 1,3-diphenyl-1,3-propanedione, ethylbenzoyl acetate, ethyl
phenylglyoxylate, 4'- hydroxypropiophenone, 1,3-indandione, 1-indanone, isopropyl
phenyl ketone, 6-methoxy-1,2,3,4- tetrahydronaphthalen-1-one, methylphenyl
glyoxylate, phenylglyoxylonitrile, 1-phenyl-1,2-propanedione 2-oxime, valerophenone,
2-acetyl- γ -butyrolactone, 2-acetylpyrrole, 1-benzylpiperidin-4-one, dehydroacetic acid,
25 3,4-dihydro-4,4-dimethyl-2H-pyran-2-one, 1,4-dihydro-4-pyridinone, N-eth-
oxycarbonyl-4-piperidinone, 2-methyl furyl ketone, 5-hydroxy-2-hydroxymethyl-4H-
pyran-4-one, 3-hydroxy-2-methyl-4-pyranone, 3-indolyl methyl ketone, isatin, 1-
methyl-4-piperidinone, methyl 2-pyridyl ketone, methyl 3-pyridyl ketone, methyl 4-
30 pyridyl ketone, methyl 2-thienyl ketone, phenyl 2-pyridyl ketone, phenyl 4-pyridyl
ketone, tetrahydrofuran-2,4-dione, tetrahydro-4H-pyran-4-one, 2,2,6,6-tetramethyl-4-
piperidone, xanthone, acenaphthene quinone, pyruvic acid, (1 R)-(-)-camphor quinone,
(IS)-(+)-camphor quinone, 3,5-diert. butyl-o-benzoquinone, 1,2-dihydroxy-3,4-
cyclobutendione, ethyl (2-amino-4-thiazolyl)glyoxylate, ethyl pyruvate, 2,3-

hexanedione, 3,4- hexanedione, 3-methyl-2-oxobutyric acid, 3-methyl-2-oxovaleric acid, 4-methyl-2-oxovaleric acid, 2- oxobutyric acid, 2,3-pentandione, 9,10-phenanthrene quinone, acetoacetanilide,2-acetyl-γ-butyrolactone, 2-acetylcylopentanone, allyl acetoacetate, benzoylacetone, tert.butyl acetoacetate, 1,3-
5 cyclopentanedione, diethyl 3-oxoglutamate, dimethyl acetylsuccinate, dimethyl 3-oxoglutarate, 1,3-diphenyl-1,3-propanedione, ethyl acetoacetate, ethyl benzoylacetate, ethyl butyrylacetate, ethyl 2-oxocyclohexanecarboxylate, ethyl 2-phenylacetacetate, methyl acetoacetate, 2-methyl-1,3- cyclohexanedione, 2-methyl-1,3-cyclopentanedione, methyl isobutyrylacetate, methyl 3-oxopentanoate, methyl pivaloylacetate, 3-
10 oxoglutaric acid, tetrahydrofuran-2,4-dione, 2,2,6,6-tetramethyl-3,5-heptanedione, 3-benzoylpropionic acid, 1,4-cyclohexanedione, dimethyl acetylsuccinate, ethyl levulinate, 2-aminoanthraquinone, anthraquinone, p-benzoquinone, 1,4-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone, 2-ethylanthraquinone, methyl-p-benzoquinone, 1,4-naphthoquinone, tetramethyl-
15 benzoquinone, 2,2-dimethyl-1,3-dioxan-4,6-dione, 2-benzoylbenzoic acid, 3-benzoylpropionic acid, 5,6-dimethoxyphthalaldehydic acid, levulinic acid, methyl trans-4-oxo-2-pentenoate, phthalaldehydic acid, terephthalaldehydic acid, dibutyl maleate, dibutyl succinate, dibutyl phthalate, dicyclohexyl phthalate, diethyl acetamidomalonate, diethyl adipate, diethyl benzylmalonate, diethyl butylmalonate, diethylmethoxymethylene-
20 malonate, diethyl ethylmalonate, diethyl fumarate, diethyl glutarate, diethyl isopropylidenemalonate, diethyl maleate, diethyl malonate, diethyl methylmalonate, diethyl oxalate, diethyl 3-oxoglutarate, diethyl phenylmalonate, diethyl phthalate, diethyl pimelate, diethyl sebacate, diethyl suberate, diethyl succinate, diisobutyl phthalate, dimethyl acetylene- dicarboxylate, dimethyl acetylsuccinate, dimethyl adipate, dimethyl 2-aminoterephthalate, dimethyl fumarate, dimethyl glutaconate, dimethyl glutarate, dimethyl isophthalate, dimethyl malonate, dimethylmethoxy-malonate, dimethyl methylenesuccinate, dimethyl oxalate, dimethyl 3-oxoglutarate, dimethyl phthalate, dimethyl succinate, dimethyl terephthalate, ethylene glycol diacetate, ethylene glycol dimethacrylate, monoethyl fumarate, monomethyl malonate, monoethyl adipate, monomethyl phthalate, monomethyl pimelate, monomethyl terephthalate, 1,2-propylene glycol diacetate, triethyl methanetricarboxylate, trimethyl 1,2,3-propanetricarboxylate, 3-acetoxy-2-cyclohexen-1-one, allyl acetoacetate, allyl
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cyanoacetate, benzyl acetoacetate, *tert*.butyl acetoacetate, butyl cyanoacetate,
chlorogenic acid hemihydrate, coumarin-3-carboxylic acid, diethyl ethoxy-
carbonylmethanephosphonate, dodecyl gallate, dodecyl 3,4,5-trihydroxybenzoate, (2,3-
epoxypropyl) methacrylate, (2-ethoxyethyl) acetate, ethyl acetamidocyanoacetate, ethyl
5 2-aminobenzoate, ethyl 3-aminopyrazol-4-carboxylate, ethyl benzoxyacetate, ethyl
butyrylacetate, ethyl cyanoacetate, ethyl 2-cyano-3-ethoxyacrylate, ethyl cyanoformate,
ethyl 2-cyanopropionate, ethyl 3,3-diethoxypropionate, ethyl 1,3-dithian-2-carboxylate,
ethyl 2-ethoxyacetate, ethyl 2-furancarboxylate, ethyl levulinate, ethyl mandelate, ethyl
gallate, ethyl 2-methyllactate, ethyl 4- nitrocinnamate, ethyl oxamate, ethyl 2-
10 oxocyclohexanecarboxylate, ethyl 4-oxocyclohexane- carboxylate, ethyl 5-
oxohexanoate, ethyl 2-phenylacetacetate, ethyl 4-piperidinecarboxylate,
ethyl 2-pyridinecarboxylate, ethyl 3-pyridinecarboxylate, ethyl 4-pyridinecarboxylate,
ethyl thioglycolate, ethyl 3,4,5-trihydroxybenzoate, 2-hydroxyethyl methacrylate, 2-
hydroxypropyl methacrylate, 3-indole acetate, 2-methoxyethyl acetate, 1-methoxy-2-
15 propyl acetate, methyl 2- aminobenzoate, methyl 3-aminocrotonate, methyl
cyanoacetate, methyl 4-cyanobenzoate, methyl 4- formylbenzoate, methyl 2-
furancarboxylate, methyl isobutyrylacetate, methyl methoxyacetate, methyl 2-
methoxybenzoate, methyl 3-oxopentanoate, methyl phenylglyoxylate, methyl phenyl-
sulfinylacetate, methyl pivatoylacetate, methyl 3-pyridinecarboxylate, 5-
20 nitrofurfurylidene diacetate, propyl gallate, propyl 3,4,5-trihydroxybenzoate, methyl 3-
methylthiopropionate, acetamide, acetanilide, benzamide, benzanilide, N,N-
diethylacetamide, N,N-dimethylformamide, N,N-diethyl-3-methyl- benzamide,
diethyltoluamide, N,N-dimethylacetamide, N,N-diphenylacetamide, N-
25 methylformamide, N-methylformanilide, N-acetylthiourea, adipic acid diamide, 2-
aminobenzamide, 4-aminobenzamide, succinic acid diamide, malonic acid diamide,
N,N'-methylene diacrylamide, oxalic acid diamide, pyrazine-2-carboxamide,
pyridine-4-carboxamide, N,N,N',N'-tetramethylsuccinic acid diamide,
N,N,N',N'-tetramethylglutaric acid diamide, acetacetanilide, benzohydroxamic acid,
30 cyanoacetamide, 2-ethoxybenzamide, diethyl acetamidomalonate, ethyl
acetamidocyanoacetate, ethyl oxamate, hippuric acid Na salt, N-(hydroxy-
methyl)acrylamide, L-(*l*)-lactamide, 2'-nitroacetanilide, 3'- nitroacetanilide, 4'-
nitroacetanilide, paracetamol, piperine, salicylanilide, 2-acetyl- γ -butyrolactone, γ -

butyrolactone, ϵ -caprolactone, dihydrocoumarin, 4-hydroxycoumarin, 2-(5H)-furanone, 2,5-dihydro-5-methoxy-2-furanone, phthalide, tetrahydrofuran-2,4-dione, 2,2,6-trimethyl-1,3-dioxin-4-one, γ -valerolactone, 4-amino-1,3-dimethyluracil, barbituric acid, O-benzyloxycarbonyl-N-hydroxysuccinimide, succinimide, 3,6-dimethyl-

5 piperazin-2,5-dione, 5,5-diphenylhydantoin, ethyl 1,3-dioxoisindoline-2-carboxylate, 9-fluorenylmethylsuccinimidyl carbonate, hydantoin, maleimide, 3-methyl-1-phenyl-2-pyrazolin-5-one, 1-methyl-2-pyrrolidone, methyluracil, 6-methyluracil, oxindole, phenytoin, 1-(2H)-phthalazinone, phthalimide, 2,5-piperazinedione, 2-piperidinone, 2-pyrrolidone, rhodanine, saccharin, 1,2,3,6-tetrahydrophthalimide, 1,2,3,4-tetrahydro-10 6,7-dimethoxyquinazolin-2,4-dione, 1,5,5-trimethyl-hydantoin, 1-vinyl-2-pyrrolidone, ditert.butyl dicarbonate, diethyl carbonate, dimethyl carbonate, dimethyl dicarbonate, diphenyl carbonate, 4,5-diphenyl-1,3-dioxol-2-one, 4,6-diphenylthieno-(3,4-d)-1,3-dioxo-2-one 5,5-dioxide, ethylene carbonate, magnesium methoxide methyl carbonate, monomethyl carbonate Na salt, propenyl carbonate, N-allylurea, azodicarbonamide, N-15 benzylurea, biuret, 1,1'-carbonyldiimidazol, N,N-dimethylurea, N-ethylurea, N-formylurea, urea, N-methylurea, N-phenylurea, 4-phenylsemicarbazide, tetramethylurea, semicarbazide hydrochloride, diethyl azodicarboxylate, methyl carbamate, 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanone and 1-(4-methoxyphenyl)-2-(2-methoxyphenoxy)ethanol.

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Also preferred are anhydrides, such as the following:

Benzoic anhydride, benzene-1,2,4,5-tetracarboxylic acid-1,2,4,5-dianhydride, 3,3',4,4'-benzophenonetetracarboxylic anhydride, succinic anhydride, butyric anhydride, crotonic

25 anhydride, cis-1,2-cyclo- hexanedicarboxylic anhydride, ditert.butyl dicarbonate, dimethyl dicarbonate, dodecenylsuccinic anhydride, Epicon B 4400, acetic anhydride, glutaric anhydride, hexanoic anhydride, isatoic anhydride, isobutyric anhydride, isovaleric anhydride, maleic anhydride, 1,8-naphthalenedicarboxylic anhydride, 3-nitrophthalic anhydride, 5-norbornene-2,3-dicarboxylic anhydride, phthalic anhydride, 30 2-phenylbutyric anhydride, pivalic anhydride, propionic anhydride, cis-1,2,3,6-tetrahydronaphthalic anhydride and valeric anhydride.

Particularly preferred are benzophenones such as the following:

Benzophenone, 4-aminobenzophenone, 2-amino-5-chlorobenzophenone, benzophenone-2-carboxylic acid, (S)-(-)-2-(N-benzopropyl)aminobenzophenone, 4,4'-5 bis(dimethylamino)benzophenone, 4,4'- bis(diethylamino)benzophenone, 3,4-dimethoxybenzophenone, 4,4'-dihydroxybenzophenone, 2,4- dihydroxybenzophenone, 4-hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 4,4'-dimethoxybenzophenone, 2,2',4,4'-tetrahydroxybenzophenone and 2-chlorobenzophenone.

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APPENDIX III

Polymerization Catalysts: Phenolic Compounds, Phenol Derivatives or Other Phenolic Polycyclic Compounds with a Number of Oxidizable Hydroxyl Groups

Preferably, such polymerization catalysts are, for example, the following:

15 alizarin, 5-amino-2-hydroxybenzoic acid, 3-aminophenol, pyrocatechol, 2,2-bis(4-hydroxyphenyl)-propane, bis(4-hydroxyphenyl)methane, quinalizarin, 4-chloro-1-naphthol, coniferyl alcohol, 2,4-di- aminophenol dihydrochloride, 3,5-dichloro-4-hydroxyaniline, 1,4-dihydroxyanthraquinone, 2,2-di- hydroxybiphenyl, 4,4-dihydroxybiphenyl, 2,3-dihydroxynaphthalene, 2,6-diisopropylphenol, 3,5-di- methoxy-20 4-hydroxybenzhydrazine, 2,5-ditert butylhydroquinone, 2,6-ditert butyl-4-methylphenol, 4-hydroxybiphenyl, 2-hydroxydiphenylmethane, 2-(2-hydroxy-phenyl)benzothiazole, 5-indanol, 2-iso- propoxyphenol, 4-isopropyl-3-methylphenol, 5-isopropyl-2-methylphenol, 4-isopropylphenol, lauryl gallate, 2-naphthol, 4-nonylphenol, 3-(pentadecyl)phenol, 2-propylphenol, 4-propylphenol, purpurine, pyrogallol, 4-25 (1,1,3,3-tetramethylbutyl)phenol, 1,2,4-trihydroxybenzene, 2,4,6-trimethylphenol, 2,3,5-trimethylphenol, 2,3,6-trimethylphenol, 3,4,5-trimethylphenol, 6,7-dihydroxy-4-methyl coumarin, 2-(2-hydroxyethoxy)benzaldehyde, 1-naphthol, nordihydroguaiaretic acid, octyl gallate, silibinin, 3,4,6-trihydroxybenzoate-octylester, 2,4,6-tritert butylphenol, 2,4-ditert butylphenol, 2,6-dichlorophenol, indophenol, ethoxyquin, 1-aminoanthraquinone, 2-amino-5-chlorobenzophenone, 4-aminodi- phenylamine, 7-

amino-4-hydroxy-2-naphthalenesulfonic acid, 2-(4-aminophenyl)-6-ethylbenzothiazole, benzanthrone, trioctyl trimellitate, trans-chalcone, bis(4-aminophenyl)amine sulfate,

2,2'- ethylenebis (4,6-ditert.butylphenol), 2,2-bis(2,6-dibromo-4-(2-hydroxy-ethoxyphenyl)propane, bis(3,5-ditert.butyl-4-hydroxyphenyl)methane, 2,2-bis(3,5-

5 dichloro-4-hydroxyphenyl)propane, Bismarck Brown Y, 1-bromophthalein, 4-butylaniline, 2-tert.butyl-5-methylphenol, 1-chloro-anthraquinone, 2-chloro-anthraquinone, triallyl 1,3,5-benzenetricarboxylate, 1,1,1-tris(hydroxy-methyl)propane, tri-methacrylate, pentaerythritol triacrylate, 1,2,4-trivinylcyclohexane, trans,cis-cyclododeca-1 5,9-triene, pentaerythritol tetrabenzoate, 4,4'-methylene-bis(2,6-ditert.butylphenol), 4,4'-isopropylidene-bis(2,6-dichlorophenol), 4,4'-isopropylidene-bis(2,6-dibromophenol), 4,4'-isopropylidene-bis[2-(2,6- dibromophenoxy)ethanol, 2,2'-ethylenidene-bis(4,6-ditert.butylphenol), 3-tert.butyl-4-hydroxy-5-methylphenol, 5-tert.butyl-4-hydroxy-2-methylphenol, syringaldazine, 4,4'-dimethoxytriphenylmethane and di-sec.butylphenol.

15 **Also particularly preferred are compounds with several hydroxyl groups, such as:** ellagic acid, gallic acid, gallein, gallangin, myoinositol, morin, nitranilic acid, phenolphthalein, purpurin, purpurogallin, quinizarin, chrysazin, quercitin, quinhydrone, chloranilic acid, carmine, rhodizonie acid, croconic acid, meilitic acid, hematoxylin, 9-phenyl-2,3,7-trihydroxy-6-fluorene, 9-methyl-2,3,7- trihydroxy-6-fluorene, 20 tetrahydroxy-p-benzoquinone, 2,2',4,4'-tetrahydroxybenzophenone, Pyragallol Red, 1-nitrophloroglucinol, 1,4-dihydroxyanthraquinone, 5,8-dihydroxy-1,4-naphthoquinone, hexa- oxocyclohexane octahydrate, 5,7-dihydroxyflavanone, 3',4'-dihydroxyflavanone, glyoxal hydrate, 1,3,5-tris(2-hydroxyethyl)isocyanuric acid, quinalizarin and 2,4,5-trihydroxybenzamine.

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APPENDIX IV

Appendix IV shows the formulas of mediators/mediation enhancers (NO₂, NOH- and HNR-OH compounds) which according to the invention can be added to the enzyme component system (ECS) together with oxidoreductases, such as, for example:

5 **Hydroxylamines (linear or cyclic, aliphatic or aromatic, heterocyclic) of general formula I)**



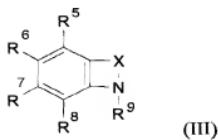
10 (I)

such as compounds of general formula II

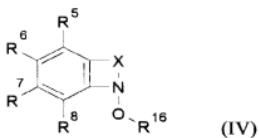


15 (II)

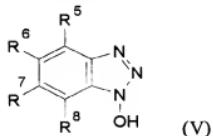
20 **such as compounds of general formula III:**



such as compounds of general formula IV:



5 **such as compounds, namely derivatives, of 1-hydroxybenzotriazole and the tautomeric benzotriazole 1-oxide, as well as the esters and salts thereof, particularly compounds of formula V :**

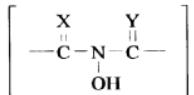


10 **such as, for example, the following compounds:**

1 -hydroxybenzotriazole
 1 -hydroxybenzotriazole, sodium salt
 15 1 -hydroxybenzotriazole, potassium salt
 1 -hydroxybenzotriazole, lithium salt
 1 -hydroxybenzotriazole, ammonium salt
 1 -hydroxybenzotriazole, calcium salt
 1 -hydroxybenzotriazole, magnesium salt
 20 1 -hydroxybenzotriazole-6-sulfonic acid, monosodium salt
 1 -methoxy- 1 H-benzotriazole
 1 -acetoxy- 1 H-benzotriazole
 1 -hydroxy-(4,5-f)-dioxolo-1 H-benzotriazole
 1 -hydroxy-6-methyl-3H-benzotriazole
 25 1-hydroxy-6-nitro-1 H-benzotriazole

1 -hydroxy-5,6-dimethyl-1 H-benzotriazole
 1 -hydroxy-6-methoxy-1 H-benzotriazole
 1 -hydroxy-5,6-dimethoxy-1 H-benzotriazole
 1 -hydroxy-1 H-benzotriazole-6-carboxylic acid
 5 1,5-dihydroxy-1 H-benzotriazole
 1 -hydroxy-1 H-benzotriazole-6-sulfonic acid hydrazide
 1 -hydroxy-1 H-benzotriazole-6-carboxamide
 1 -hydroxy-5-methoxy-1 H-benzotriazole
 6-amino-1 -hydroxy-1 H-benzotriazole
 10 6-amino-5-methoxy-1 H-benzotriazole
 6-chloro- 1 -hydroxy- 1 H-benzotriazole
 6-acetamido-1 -hydroxy-1 H-benzotriazole
 1 -hydroxy- 1 H-benzotriazole-6-carboxylic acid ethyl ester
 1 -hydroxy-4-nitro-1 H-benzotriazole
 15 4-chloro-1 -hydroxy-1 H-benzotriazole
 1 -hydroxy-6-tert. butyl- 1 H-benzotriazole
 6-cyclohexyl-1 -hydroxy-1 H-benzotriazole
 6-isopropyl-1 -hydroxy-1 H-benzotriazole
 1 -hydroxy-6-phenyl-1 H-benzotriazole
 20 3-methyl-3H-benzotriazole 1 -oxide
 2-phenyl-2H-benzotriazole 1 -oxide

such as compounds of general formula A (cyclic N-hydroxy compounds):

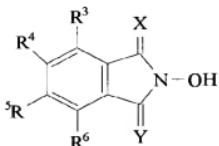


(A)

25

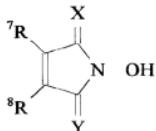
Such as compounds of general formula VI, VII, VIII or IX:

5



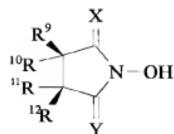
VI

10

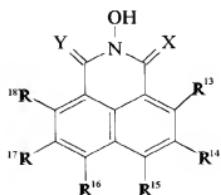


VII

15



VIII



IX

20

for example, compounds such as:

N-hydroxyphthalimide and optionally substituted N-hydroxyphthalimide derivatives, N-hydroxymaleimide and optionally substituted N-hydroxymaleimide derivatives, N-

hydroxynaphthalimide and optionally substituted N-hydroxynaphthalimide derivatives, N-hydroxysuccinimide and optionally substituted N-hydroxysuccinimide derivatives, **such as, for example:**

5 N-hydroxyphthalimide, N-hydroxybenzene-1,2,4-tricarboximide,
 N,N'-dihydroxypyromellitic acid diimide,
 N,N'-dihydroxybenzophenone-3,3',4,4'-tetracarboxylic acid diimide,
for example, (formula VII):

10 N-hydroxymaleimide,
 pyridine-2,3-dicarboxylic acid N-hydroximide

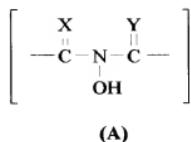
for example (formula VIII):

15 N-1-hydroxysuccinimide, N-1-hydroxytartarimide,
 N-hydroxy-5-norbornene-2,3-dicarboximide,
 exo-N-hydroxy-7-oxabicyclo[2.2.1]-5-heptene-2,3-dicarboximide,
 N-hydroxy-cis-cyclohexane-1,2-dicarboximide,
 N-hydroxy-cis-4-cyclohexene-1,2-dicarboximide

20
for example (formula IX):

N-hydroxynaphthalimide sodium salt

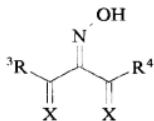
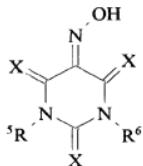
25 **for example (six-membered ring of formula A):**



10 N-hydroxyglutarimide

such as compounds of general formula X or XI (oximes):

5

**X****XI**

10

for example (formula X):

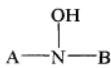
dimethyl 2-hydroxyiminomalonate

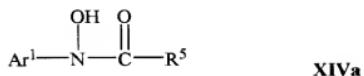
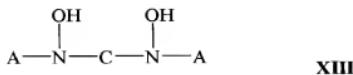
15

for example (formula XI):

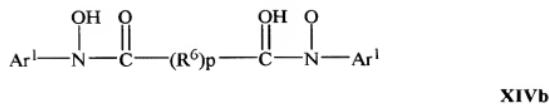
1-methylvioluric acid, 1,3-dimethylvioluric acid, thiovioluric acid, alloxane 4,5-dioxime, alloxane 5-oxime hydrate (violuric acid) and/or the esters or salts thereof,

20 such as compounds from the class of N-aryl-N-hydroxyamides of general formula XII, XIII and XIV, XIVa, XIVb, XIVc, XIVd and XIVe:

**XII**



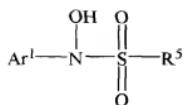
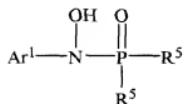
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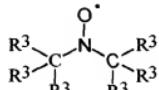
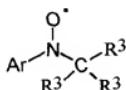
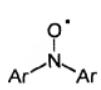


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**XIVd****XIVe**

5

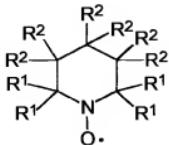
10 such as compounds of general formula **XV**, **XVI** and **XVII** (nitroxyl radicals/nitroxides)

**XV****XVI****XVII**

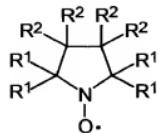
15

such as compounds of general formula XVII a and XVII b (nitroxyl radicals):

5



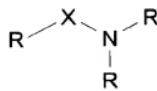
XVIIa



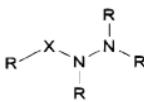
XVIIb

10 Such as compounds of general formula XVIIIa (amides) and XVIII b (hydrazides):

15



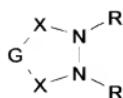
XVIIIa



XVIII b

such as compounds of general formula XVIII c (cyclic hydrazides):

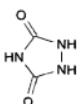
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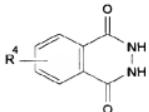
XVIII c

such as urazoles (formula XVIII d) and phthalhydrazides (formula XVIII e):

5



XVIII d

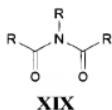


XVIII e

10

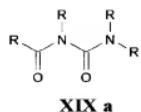
such as compounds of general formula XIX (imides):

15



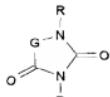
XIX

such as compounds of general formula XIXa (imides):



XIX a

such as compounds of general formula XIXb (cyclic imides):

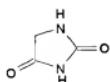


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XIX b

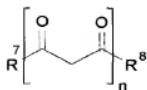
such as compounds of general formula XIXc (hydantoin derivatives):

10

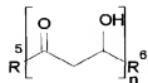
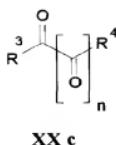
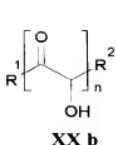
**XIX c**

15 such as compounds of general formula XX, such as α -hydroxycarbonyl compounds of general formula XXa, α -dicarbonyl compounds of general formula XXb, β -hydroxycarbonyl compounds of general formula XXc and β -dicarbonyl compounds of general formula XXd:

20

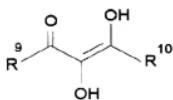
**XX a**

25



5 such as compounds of general formula XXI (linear compounds with double bonds/enols):

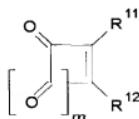
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XXI

15 such as compounds of general structure XXII (cyclic compounds, groups not OH, derivatives of squaric acid, OH group derivatized):

20



such as, for example:

deltic acid, squaric acid, croconic acid and rhodizonic acid

5

*The formula descriptions (groups/R ... are given in patent application DE 197 19 857.0.

10 **APPENDIX IVa**

Appendix IVa shows compounds which can be added to the enzyme component system (ECS) of the invention as mediation enhancers, primarily as additives together with mediators and oxidoreductases:

15

Aliphatic ethers and aryl-substituted alcohols, such as:

2,3-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol, 2,4-dimethoxybenzyl alcohol, 2,6-dimethoxybenzyl alcohol, homovanillic alcohol, ethylene glycol

20

monophenyl ether, 2-hydroxybenzyl alcohol, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol, 2,5-dimethoxybenzyl alcohol, 3,4-dimethoxybenzylamine, 2,4-dimethoxybenzylamine hydrochloride, veratryl alcohol, coniferyl alcohol.

25

olefins (alkenes), for example:

2-allylphenol, 2-allyl-6-methylphenol, allylbenzene, 3,4-dimethoxypropenylbenzene, p-methoxystyrene, 1-allylimidazole, 1-vinylimidazole, styrene, stilbene, allyl phenyl ether, benzyl cinnamate, methyl cinnamate, 2,4,6-triallyloxy-1,3,5-triazine,

1,2,4-trivinylcyclohexane, 4-allyl-1,2-dimethoxybenzene, 4-tert. benzoic acid vinyl ester, squalene, benzoin allyl ether, cyclohexene, dihydropyran and N-benzylcinnamanilide.

Phenol ethers, such as:

5

2,3-dimethoxybenzyl alcohol, 3,4-dimethoxybenzyl alcohol,
2,4-dimethoxybenzyl alcohol, 2,6-dimethoxybenzyl alcohol, homovanillic alcohol, 4-hydroxybenzyl alcohol, 4-hydroxy-3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol, 2,5-dimethoxybenzyl alcohol, 3,4-dimethoxybenzylamine, 2,4-dimethoxybenzylamine
10 hydrochloride, veratryl alcohol, coniferyl alcohol, veratrol, anisole.

Carbonyl compounds, such as:

4-aminobenzophenone, 4-acetyl biphenylbenzophenone, benzil, benzophenone

15

hydrazone, 3,4-dimethoxybenzaldehyde, 3,4-dimethoxybenzoic acid, 3,4-dimethoxybenzophenone, 4-dimethylaminobenzaldehyde, 4-acetyl biphenyl hydrazone, benzophenone 4-carboxylic acid, benzoylacetone, bis(4,4'-dimethylamino)benzophenone, benzoin, benzoin oxime, N-benzoyl-N-phenylhydroxylamine, 2-amino-5-chlorobenzophenone, 3-hydroxy-4-

20

methoxybenzaldehyde, 4-methoxybenzaldehyde, anthraquinone-2-sulfonic acid, 4-methylaminobenzaldehyde, benzaldehyde, benzophenone-2-carboxylic acid, 3,3',4,4'-benzophenonetetracarboxylic dianhydride, (S)-(-)-2-(N-benzylpropyl)-aminobenzophenone, benzylphenylacetanilide, N-benzylbenzanilide, 4,4'-bis(dimethylamino)thiobenzophenone, 4,4'-bis(diacetylamino)benzophenone,

25

2-chlorobenzophenone, 4,4'-dihydroxybenzophenone, 2,4-dihydroxybenzophenone, 3,5-dimethoxy-4-hydroxybenzaldehyde hydrazine, hydroxybenzophenone, 2-hydroxy-4-methoxybenzophenone, 4-methoxybenzophenone, 3,4-dihydroxybenzophenone, p-

anisic acid, p-anisaldehyde, 3,4-dihydroxybenzaldehyde, 3,4-dihydroxybenzoic acid,

30

2,5-dimethoxy-4-hydroxybenzaldehyde, 3,5-dimethoxy-4-hydroxybenzoic acid, 4-hydroxybenzaldehyde, salicylaldehyde, vanillin, vanillic acid.

APPENDIX 5**Possible Oxidation Reactions of the Enzyme Component System****1) Hydroxylation reactions**

- 5 a) Synthesis of alcohols
- b) Hydroxylation of steroids
- c) Hydroxylation of terpenes
- d) Hydroxylation of benzenes
- e) Hydroxylation of alkanes
- 10 f) Hydroxylation of aromatic compounds
- g) Hydroxylation of double bonds
- h) Hydroxylation of nonactivated methyl groups
- i) Dihydroxylation of aromatic compounds

15 2) Oxidation of unsaturated aliphatics

- a) Preparation of epoxides
- b) Preparation of compounds by epoxidation
- c) Preparation of arene oxides
- d) Preparation of phenols
- 20 e) Preparation of cis-dihydrodiols

3) Baeyer-Villiger oxidations

- a) Baeyer-Villiger conversion of steroids

4) Oxidation of heterocycles

- a) Transformation of organic sulfides
- 25 b) Oxidation of sulfur compounds

- c) Oxidation of nitrogen compounds (formation of N-oxides etc.)
- d) Oxidation of other heteroatoms

5) Carbon-carbon dehydrogenation

- 5 a) Dehydrogenation of steroids

6) Other oxidation reactions

- a) Oxidation of alcohols and aldehydes
- b) Oxidation of aromatic methyl groups to aldehydes
- c) Oxidative coupling of phenols
- 10 d) Oxidative degradation of alkyl chains (β -oxidation etc.)
- e) Formation of peroxides or percompounds
- f) Initiation of free-radical induced chain reactions.

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